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ABSTRACT

This resource guide presents subject matter organized in outline form for four topical areas: introductory microbiology; medical microbiology; microbial genetics; and microbial physiology. The first two units comprise the two most frequently taught microbiology courses in community and junior colleges. The outlines for microbial genetics and microbial physiology present the instructor with more detailed information in basic subject areas that are reportedly more difficult to teach. The microbial genetics and physiology units are cited extensively throughout the introductory and medical units to assist the instructor with the presentation of these subjects. The outlines are not intended for student use but as background information for instructors and as a guide for developing courses of instruction. The format of each unit contains essential information, enriching information serving as illustrative materials to facilitate learning, and practical activities including illustrative materials. (Author/JN)

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Topic Outlines In Microbiology

AN INSTRUCTOR'S GUIDE FOR JUNIOR AND COMMUNITY COLLEGES

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Preface

In 1975, the American Society for Microbiology surveyed the needs of community and junior college teachers of microbiology. The study revealed that instructional materials specifically designed for these instructors were inadequate. As a result, American Society for Microbiology, through its Board of Education and Training, applied for and received a grant from the National Science Foundation to produce Topic Outlines in Microbiology. This publication is the result. Its objective is to organize bodies of scientific knowledge so that community and junior college instructors, with differing aims and training, may extract portions according to their instructional needs. The material was prepared with the support of National Science Foundation grant no. SED77-18459. However, any opinions, findings, conclusions, or recommendations expressed herein are those of the author(s) and do not necessarily reflect the views of National Science Foundation.

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Special thanks are due to those institutions who gave so generously of the time of their faculty. Without this cooperation the authors' work would not have been possible.

Appreciation is due to all those members of the scientific community who served as formal or informal evaluators of the various drafts of this document.

Finally, thanks should be given to all contributing authors. They have given of their time in the belief that the resulting publication will fill a gap in the instructional materials currently available to community and junior college instructors.

How to Use This Book

This book presents subject matter organized in outline form. The material is intended for two purposes: (1) as background information for instructors, and (2) as a guide for developing courses of instruction.

The user of this material must understand that the outlines are not intended for use by students, nor should they be used in their entirety. Rather, the instructor should *select* from the material presented here to meet his or her particular needs.

The Topic Outline is organized in four sections: Introductory Microbiology, Medical Microbiology, Microbial Genetics, and Microbial Physiology. The first two units comprise the two most frequently taught microbiology courses in community and junior colleges. The outlines for Microbial Genetics and Microbial Physiology were prepared to present the instructor with more detailed information in these basic subject areas that are reportedly difficult to teach. The Microbial Genetics and Physiology units are cited extensively throughout the Introductory and Medical units to assist the instructor with the presentation of these subjects.

Each unit is organized by the following format:

ESSENTIAL INFORMATION

A body of knowledge that the authors believe is essential subject matter.

ENRICHING INFORMATION

Information that the authors believe will serve as illustrative material and which will facilitate learning.

PRACTICAL ACTIVITIES

Activities allowing the instructor to enhance the use of the essential and enriching information. Illustrative materials are also included.

Fundamental to this approach is the concept that even though the authors have organized the materials in one fashion, the instructor has the option of reorganizing it in another. What may be deemed enriching at one stage of a student's progress may become essential knowledge at a later date. Similarly, information that is essential to a student with one educational aim may be enriching to another whose educational aim is different.

Resources

The user of this book may find a need for additional sources of information concerning microorganisms and the microbial world. Many are available including textbooks, laboratory manuals, magazine and journal articles, and audiovisual collections. For further information, you may wish to consult a local college or university, health department, or a public or special library. Scientific supply and equipment firms (the manufacturing industry), as well as audiovisual and publishing distributors, are often useful sources for information.

Titles of textbooks may be found in *Books in Print*. Reviews of textbooks periodically appear in such publications as *ASM News*. *The American Scientist*, *Bioscience*, *Journal of Junior College Science Teaching*, and *The Quarterly Review of Biology*.

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TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
1.0 General Introduction			
1.1 The Nature of Microorganisms	Microorganisms are microscopic and submicroscopic forms having characteristics of living systems. Viruses, bacteria (including the rickettsias and chlamydias), most fungi, protozoans, and certain algae represent the five general forms of microorganisms.	The smallest organism that can be seen with the naked eye would be approximately 0.1 mm. The smallest organism that can be seen using a light microscope would be approximately 0.2 μm (1 μm = 10^{-6} m). As a rule, viruses are the smallest, followed by bacteria, fungi, and certain algae. Microorganisms have a wider range of physiological potentialities than all other organisms combined. All microorganisms have some means of reproducing or increasing their numbers. Microorganisms play a significant role in most areas of human activity.	Most microorganisms can be fixed (immobilized) on slides and stained with various aniline dyes to demonstrate specific structures and other distinguishing properties. Compound light microscopes are used to observe most microorganisms in various preparations.
1.2 Concept of the Cell	With the exception of viruses (which are acellular), the cell is the basic structural unit of life. Most microorganisms are unicellular organisms.	Cells are composed of protoplasm (a colloidal organic complex consisting largely of proteins, lipids, and nucleic acids) surrounded by limiting membranes and containing a nucleus or nucleoid. Many cells have cell walls in addition to limiting membranes. Viruses lack the typical structures (cell wall, cytoplasmic membrane, cytoplasm, and organelles) and metabolic processes normally associated with living cells. Most microorganisms are biologically and biochemically independent cells, and they can reproduce independently in a suitable environment. A single-celled organism is able to carry out all the processes associated with life within one cell; thus, microorganisms are quite complex physiologically despite their small size. Unicellular organisms can aggregate to form multicellular units, e.g., chains or filaments of bacteria.	Staining procedures may be used to demonstrate basic structural units of life, such as the cell wall and cell membrane alone, protoplasm, and nuclear material (chromosomes).
1.3 Eucaryotic and Procaryotic Microorganisms	Two major types of cellular organization, procaryotic and eucaryotic, are recognized.	Procaryotic microorganisms lack a defined nucleus with typical nuclear membranes. Although the nuclear material is separated from the cytoplasm, it may not be visible with the light microscope even when using special stains.	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		Eucaryotic organisms possess a defined membrane-bound nucleus, which may be visible with the light microscope in either stained or unstained preparations.	
1.31 Comparison of Cell Structures	Based on the detailed organization of their cellular structures, eucaryotic microorganisms are more complex.	Distinguishing procaryotic and eucaryotic features are summarized in Table 1. Many of the functions performed by various membrane-bound organelles in eucaryotic cells are associated with the cytoplasmic membrane in procaryotes.	With the exceptions of the nucleus and the chloroplast, most eucaryotic organelles are not visible with light microscopy, but they are visible with electron microscopy.
	Bacteria (including the blue-greens or cyanobacteria) are procaryotic, whereas fungi, protozoans, and algae are eucaryotic.	Most bacteria, including cyanobacteria (formerly known as blue-green algae), are unicellular and lack an organized nucleus. Cyanobacteria contain photosynthetic apparatus (thylakoid) with chlorophyll *a*. Other photosynthetic bacteria lack chlorophyll *a*, but contain bacteriochlorophylls *a*, *b*, *c*, and *d*.	Prepared microscope slides may be used to show size relationships among various microorganisms.
	Viruses are neither procaryotic nor eucaryotic, since they lack a true cellular organization.	Viruses replicate only in living cells. Viral groups may be differentiated on the basis of the type of nucleic acid core. Viruses contain DNA or RNA, but never both.	Typical electron micrographs may be examined to show that viruses lack a cell wall, cell membrane, and cytoplasm and contain a nucleic acid core.

TABLE 1. *Features distinguishing procaryotic and eucaryotic cells*

Feature	Procaryotic	Eucaryotic
Nuclear body	Not bounded by a nuclear membrane	Bounded by a nuclear membrane
	One circular chromosome	One or more linear chromosomes
	No mitotic or meiotic division	Mitotic and meiotic division
	Nucleolus absent	Nucleolus present
Cytoplasmic nature and structures		
Cytoplasmic streaming	Absent	Present
Pinocytosis	Absent	Present
Internal membrane-bound organelles	Absent	Present
Mitochondria	Absent	Present
Golgi apparatus (dicytosomes)	Absent	Present
Endoplasmic reticulum	Absent	Present
Chloroplasts	Absent	May be present, depending on type of organism
True vacuoles	Absent	Present
Cell wall	Presence of peptidoglycan	Absence of peptidoglycan
Locomotor organelles	Simple and noncontractile	Multifibrilled and contractile

	TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
2.0	**Survey of the Microbial World**			
2.1	Classification	Biological classification (taxonomy) is the systematic arrangement of organisms in groups or categories (taxa).	One of the primary criteria used in classification is the mode of obtaining carbon (autotrophic or heterotrophic). Autotrophs and heterotrophs can be further divided into groups according to their source of energy. Additional classification criteria include: ability to grow in the presence of free oxygen (aerobic and anaerobic), guanine-cytosine (G-C) ratio, and in some instances chemical composition of the cell wall and means of motility. In the case of bacteria, serological (immunological) classification can be used where morphology, physiology, and other properties of two bacteria are similar. Organisms identified by serological means are referred to as serotypes.	Bacteria can be classified on the basis of several demonstrable characteristics, including cell shape (coccus, rod, spirillum), Gram stain reactions, photosynthesizing capability, and the ability to grow in different media.
2.11	Nomenclature	Nomenclature refers to the terms used in the systematic and scientific naming of organisms.		
2.12	Binomial System	All microorganisms except viruses have at least two names: genus and species (binomial system of nomenclature).	The naming of bacteria, for example, is governed by an internationally accepted set of rules (*International Code of Nomenclature of Bacteria*). A genus represents a group of very closely related species. A species represents one kind of microorganism capable of interbreeding with one of its own kind only or descendants from a single cell line. The names of both genus and species are Latinized and are descriptive of an organism's properties, e.g., *Micrococcus* (a small grain), *albus* (white), therefore, a small, spherical organism which produces white colonies. The first name of an organism is the genus; the second is the species. The first letter of the genus is capitalized, whereas the first letter of the species is not. Both designations are italicized or underlined.	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
2.2 The Five-Kingdom Approach of Whittaker (Note: other approaches exist.)	Based on their characteristics, organisms are grouped into the following five kingdoms: Animal, Plant, Fungi, Protista, and Monera.	The five kingdoms are differentiated primarily on the basis of the mode of obtaining energy, morphology (simple or complex), and motility. The Plant and Animal Kingdoms are differentiated by the presence or absence of photosynthetic pigments, i.e., chlorophylls *a*, *b*, and *c*. Most animals are motile and lack cell walls, whereas most higher plants are nonmotile and possess cell walls containing cellulose and fungi and lack chlorophylls. Some Protista (algae) are more closely related to plants, whereas certain other Protista (protozoans) are more closely related to the animals.	Microscope slides can be used to demonstrate the different organisms found in Protista and Monera. **Waterbury, J. B., and R. Y. Stanier.** 1978. Patterns of growth and development in pleurocapsalean cyanobacteria. Microbiol. Rev. **42**:2–44.
2.21 The Animal Kingdom	The Animal Kingdom includes organisms that are multicellular, eucaryotic, and nonphotosynthetic; that lack cell walls; and that have tissue differentiation and holozoic nutrition (capable of ingesting entire particles or whole organisms).	The tissues of higher animals are grouped into organs and systems. Most higher animals reproduce primarily by sexual means. Most animals usually ingest solid food (holozoic), which is broken down into smaller components by enzymatic activity in the digestive tract.	Most animals may be shown to respond to external stimuli (irritability). Examination of either living or preserved specimens may show that most animals possess a digestive cavity.
2.22 The Plant Kingdom	The Plant Kingdom includes organisms that are multicellular, eucaryotic, and photosynthetic and have tissue differentiation and holophytic (absorptive) nutrition.	Tissue differentiation in higher plants begins with parenchymal cells, which may give rise to supporting and conducting tissues, i.e., tracheary elements. Some plant cells are nonliving at maturity but still carry out specific functions, e.g., vascular and supportive plant tissue.	Examination of either living or preserved specimens will show that most higher plants are differentiated into tissues and true roots, stems, and leaves which are composed of specialized cells and tissues; lower plants lack true roots, stems, and leaves, but may be multi-cellular with little differentiation in cell types.
2.23 The Fungi	Fungi are achlorophyllous, unicellular, and/or multicellular and exhibit holophytic nutrition.	Most fungi are chemoorganotrophic (heterotrophic) and aerobic saprophytes; however, some are parasitic and pathogenic. All fungal class members except the Deuteromycetes (Fungi Imperfecti) can reproduce sexually as well as asexually. The hyphae of some fungi are nonseptate, whereas the majority are septate. Aggregates of hyphae are referred to as mycelia. Diseases produced by fungi may be di-	Media such as Sabouraud's dextrose agar can be used to show that fungi are acidophilic, i.e., grow better in an environment with a low pH. Prepared microscope slides may be used to show cellular features of fungi.

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			vided into two types, superficial mycoses and systemic mycoses (Medical Microbiology, Topic 16). Some fungi produce powerful toxins (mycotoxins, e.g., aflatoxin) (Medical Microbiology, Subtopic 7.82).	
2.24	The Protista	The Protista consist of eucaryotic, predominately unicellular organs that include algae and protozoans.	The algae excluded from the Protista are multicellular and display cellular differentiation with complex morphology, i.e., holdfast, stipe, and blade (which have some resemblances to root, stem, and leaves). Some protozoans have either ingestive or phagotrophic nutrition. All are heterotrophic and most are motile in at least one stage of their life cycle. Most Protista are predominately aquatic in habitat, but some are able to grow and multiply in moist soil. Some Protista possess characteristics of algae and protozoans, e.g., *Euglena* and slime molds, and generally lack rigid cell walls.	Living material or prepared microscope slides may be used to demonstrate that the Protista contain the two groups, the microscopic algae, and the protozoans. Laboratory cultures may be used to demonstrate the growth and reproductive properties of Protista.
2.25	The Monera	The Monera consist of unicellular procaryotic organisms, namely all bacteria which include the blue-greens or cyanobacteria.	Autotrophic Monera may be divided into photoautotrophs and chemoautotrophs. Photoautotrophs are divided into two groups: those able to carry out anoxygenic photosynthesis (not producing oxygen) and those able to carry out oxygenic photosynthesis (producing oxygen). Most chemoautotrophs (lithotrophs) are able to obtain energy from the oxidation of H_2, NO_2, NH_4^+, H_2S. Many heterotrophic Monera utilize organic substances as a source of energy as well as a source of carbon (Microbial Physiology, Subtopics 2.1, 2.21, and 5.23). Some heterotrophs are obligately parasitic and pathogenic (Medical Microbiology, Topic 15).	Autotrophic members of the Monera may only be grown in inorganic media, whereas heterotrophs must have organic substances for cultivation. Microscope slides prepared from living cultures or photomicrographs may be used to show various properties of the Monera.
3.0	**Microscopy and Staining**			
3.1	Microscopes	Microscopes are of two categories, light	Resolving power, or the ability to show	Diagrams may be used to show that

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	and electron, depending upon the principle on which the magnification is based.	detail, is the true measurement of any microscope. It is inversely proportional to the wavelength of the illumination source used. Since electrons have a much shorter wavelength than visible light, electron microscopes are capable of much greater resolution. A functional bright-field microscope should have the capability of resolving objects that are 0.2 μm in diameter. Electron microscopes differ in several respects from light microscopes. A series of electromagnetic lenses is used as a condenser system to concentrate the source of illumination, electrons, which have the wavelength of 0.05 Å (10^{-4} μm). Transmission electron microscopes (TEM) not only are capable of better resolving power, but are also capable of producing useful magnification ranging from 100,000 to over 500,000. An SEM can resolve objects 0.01 to 0.02 μm in diameter and has approximately five times the depth of field obtainable with a TEM. The SEM can be used to determine size, shape, and surface features of specimens.	bright-field or light microscopes used to observe biological forms are composed of a series of lenses and are called compound microscopes and that there are two types of electron microscopes, transmission (TEM) and scanning (SEM). Both electron microscopes use electrons rather than visible light as the source of illumination.
3.11 Choice of Microscope	The choice of the microscope to use depends on the nature of the specimen and the information required.	The bright-field or light microscope usually is used for the examination of living and stained preparations, e.g., Gram stains, capsule stains, and spore stains, etc. Dark-field microscopy can be employed for the detection of microbial motility and the presence of microorganisms in various specimens. Biological preparations (generally nonconductors) studied by electron microscopy must be treated in a manner to preserve structure and to distinguish them from their respective background surfaces. Intracellular submicroscopic organelles can only be seen with the TEM and requires specially prepared specimens.	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		Preparation techniques for electron microscopy include shadow-casting, freeze-etching, surface impressions or replicas, electron staining, and ultrathin sectioning (slicing).	
3.12 Advantages of Specific Types of Microscopes	Each type of microscopy and each method of specimen preparation offer some advantage in the demonstration of microscopic structures and other features of microorganisms.	Dark-field microscopy is useful in observing very fragile, difficult to stain organisms, (such as spirochetes), as well as certain selected cellular activities. Fluorescent microscopy has been used extensively for purposes of disease diagnosis and detection of proteins such as immunoglobulins. Fluorescent dyes are attached (coupled) to antibodies or immunoglobulins which combine only with the specific antigens, or substances that caused their information. Resulting colors will vary depending on the type of fluorescent dye used and the structures stained.	Preparations of certain bacteria which are difficult to see with ordinary light microscopy may be treated with fluorescent dyes and exposed to ultraviolet light to make them visible.
3.13 Bright-Field Microscopy	The bright-field compound microscope is one of the most commonly used instruments in the microbiology laboratory.	Most bright-field microscopes can be calibrated with stage and ocular micrometers. Phase-contrast can be obtained with a special optical device to enhance differences in refractive index and density of the specimen.	Many microorganisms may be seen with the bright-field compound microscope.
3.2 Staining Techniques	A variety of colored organic compounds (dyes) and staining procedures are used in the study and categorization of microorganisms.	Most dyes are basic and, therefore, have a strong affinity for the acidic components of cells, e.g., nucleic acids. Some dyes, when used in combination with other reagents, may form a complex and may be retained by the bacterial cell. Many dyes also may be used to inhibit certain microorganisms. Some of these dyes may be added to media (selective media) or used for the treatment of certain diseases.	Bacteria may be stained by techniques with single dyes known as simple staining procedures or techniques with combinations of dyes and with or without the use of decolorizing agents, which are referred to as differential staining procedures.
3.21 Major Differential Staining Procedures	The use of staining procedures enables the individual to observe cells and their respective parts more distinctly and to distinguish among certain cells and/or their associated structures.		Differential procedures may be used to separate microorganisms into specific groups, e.g., Gram stain and acid-fast; to promote visibility of structures such as spores, flagella, and capsules; and to aid in microbial identification.

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
3.22	The Gram Stain	On the basis of Gram staining results, all bacteria may be divided into two groups, gram positive and gram negative. Various factors may cause a gram-positive organism to stain gram negatively; these include aging, injury to the cell wall, and cultivation in an acidic medium.	Gram-negative organisms may exhibit a gram-positive reaction only with improper staining. The acid-fast stain is designed to detect microorganisms possessing substances (hydrophobic) on the cell wall, e.g., *Mycobacterium tuberculosis*.	
3.23	Other Differential Staining Procedures	Other differential staining procedures for such structures as spores, flagella, and capsules can be used in the identification of bacteria.	Endospores generally resist ordinary staining procedures. Application of heat during spore staining is necessary for stain penetration to occur. Since bacterial flagella are so thin (0.02 to 0.028 μm) they can be demonstrated only by the depositing of dye particles onto their surfaces. With capsule stains using India ink, it is the background surrounding the structure that is stained rather than the capsule itself.	
4.0	**Microbial Cultivation**			
4.1	The Nature of Media	The media used in the growth of microorganisms can be subdivided into two groups: complex and synthetic. Complex media are those for which the chemical composition is not defined, e.g., potato, blood, milk, meat extract, etc. Media must fulfill the nutritional requirements of microorganisms.	All complex media are not applicable for the cultivation of all types of microorganisms. Natural media such as milk and serum can be sterilized by special techniques and apparatus. Filtration is an example of a procedure used for the sterilization of natural media as well as heat-labile material. Microorganisms requiring complex or special sources of growth factors are referred to as nutritionally fastidious organisms, e.g., parasitic and certain pathogenic organisms. The selection of a medium depends on the needs of the organism to be cultured. An energy source is needed for all media except those preparations used by photoautotrophs. The nitrogen source may be inorganic (NH_4^+, NO_3^-) or organic (proteins and related compounds) or both, depending on the organism. The carbon source may be inorganic (CO_3^{2-}, HCO_3^-) or organic compounds such as lipids, carbohydrates, or proteins.	

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TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			Some organisms may utilize inorganic sulfur, whereas others require organic sulfur such as sulfur containing amino acids (methionine, cysteine, cystine).	
4.2	Media Usage	Media may be used for a variety of purposes, including bioassay, identification and isolation of particular organisms, and antibiotic susceptibility testing.	Various dyes, antibiotics, and other material are incorporated into media to inhibit or favor the growth of certain microorganisms. pH indicators are added to media to detect acid or alkaline states. Eh indicators are utilized to detect aerobic or anaerobic conditions. A variety of specific compounds (substrates) may be added to media to detect enzymatic activities and patterns of microorganisms. Media may be liquid (broth), semisolid, or solid.	Media applications include selective media for the isolation of specific organisms, e.g., Sabouraud's agar, tomato juice, and antibiotic-containing preparations; differential media to distinguish among similar organisms, e.g., blood agar; selective and differential media preparations having the combined properties of differential and selective media, e.g., eosin methylene blue agar; enrichment media to promote the growth of one type of organism over others, e.g., preparations for various autotrophs; and all-purpose media which support the growth of a wide variety of organisms, e.g., nutrient agar.
5.0	**Systematic Study of the Microbial World**			
5.1	Bacteria	The bacteria are procaryotic, unicellular organisms. They are structurally simple, and on the basis of cell shapes at least three broad groups are recognized: bacilli (rods), cocci (spherical and ovoid), and spirilla (spiral and corkscrews).	Bacterial structures such as endospores, capsules, and flagella are not found with all kinds of bacteria. Endospores are structures that enable cells to withstand adverse conditions, capsules are structures that enable some cells to avoid phagocytosis, and flagella are organelles of locomotion.	Bacteria may be cultivated in the laboratory on various kinds of media or in an appropriate kind of tissue culture system. The size, shape, and cellular arrangement of various kinds of bacteria may be determined when wet mounts and/or heat-fixed smears are observed under a compound microscope.
5.11	Gram Reactions	Bacteria can be divided into two categories on the basis of their reaction to the Gram stain procedure: gram-positive and gram-negative.		
5.12	Cell Structure	Bacterial cells do not contain discrete membrane-bound structures.	Bacterial enzymes that generate energy are located on membranes that are not organized into discrete organelles. Photosynthetic bacteria contain pigment centered on inner membranes that absorb light energy of a different wavelength than the photoreceptors of eucaryotic plants.	TEMs of bacterial cells may be used to demonstrate a granulated cytoplasm and dense areas (called a nuclear region), and the conspicuous absence of membrane-bound structures.

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
5.13	Chromosome Structure and Function	Bacteria have a single, circular chromosome. Each bacterial chromosome contains many genes, each of which codes for entire protein subunits.	Internal membranous structures, mesosomes, are believed to function during cell division through the replication of the bacterial chromosome, cell wall formation, and the synthesis of certain proteins and other compounds.	TEMs of a bacterial chromosome may be used to show that it is a circular looped-like structure in the cytoplasm that is not encircled by a membrane.
5.14	Method of Reproduction	Bacteria do not carry out the processes of mitosis or meiosis. Most bacteria reproduce through asexual processes in which the parent cell is divided equally between two progeny cells. Bacterial cells contain only identical copies of each gene after asexual reproduction. As a result of this phenomenon, they always exist in the haploid state (1N).	Certain kinds of bacteria possess sex pili, structures that function in gene transfer from donor to recipient cells.	
5.2	Fungi	Fungi are organisms that utilize only organic substances as a carbon source (heterotrophic nutrition).	Fungi contribute significantly to soil fertility by releasing small organic molecules to ecosystems from the degradations of biomass. Certain fungi degrade and obtain utilizable constituents from plastics, crude oil, paints, leather, etc.	Media containing substances such as starch, gelatin, or lipids may be used to show that fungi excrete a wide variety of hydrolytic enzymes.
5.21	Activities and Associations	These organisms lack chlorophyll and other photosynthetic pigments.	Some fungi can live optimally in extreme environments as free-living entities and absorb minerals and nutrients from rocks and other substrates that cannot be utilized by other heterotrophs. Certain fungi have characteristics that enable them to live mutualistically with higher plants (mycorrhizae) or algae (lichens). Fungi that are parasitic obtain their nutrients from other living organisms. Pathogenic fungi produce two general categories of infections in humans: those primarily associated with the skin and its appendages (dermatomycoses); and those that involve various organs of the body (systemic mycoses). Fungi such as *Aspergillus fumigatus* cause opportunistic infections. In addition, *A. flavus* and other organisms produce aflatoxins.	Field samples of fungi may be used to show that these organisms, which are saprophytes, obtain their nutrients from dead or decomposing organic matter.

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
5.22	Structure and Organization	Fungi have discrete membrane-bound organelles.	Molds may have multinucleated hyphae with cross walls (septate) or without cross walls (nonseptate) and produce various kinds of spores that are characteristic of a particular taxonomic class. Yeasts are generally nonfilamentous, but some yeasts have a filamentous phase. Dimorphic fungi, when subjected to a specific environmental change, exhibit both a yeast phase and a mycelial phase.	TEMs may be used to show the presence of various kinds of membrane-encircled structures, and that chloroplasts are absent.
5.3	Protozoans	Protozoans are predominately unicellular organisms that lack cell walls and exhibit some form of locomotion during their lifetimes.	Types of organelles of locomotion are usually characteristic for protozoan groups. Many types of protozoans are motile only during certain stages. (Introductory Microbiology, Topic 10). Certain protozoan cells assimilate inorganic chemicals such as calcium and silicon into their outermost structure. Such substances tend to give cells rigidity. The variety of shapes exhibited by certain kinds of protozoans can be attributed to features of growth periods. Protozoans that concentrate inorganic substances in their shells become temporary reservoirs of those substances and play a role in geochemical cycles. The variety of morphological forms exhibited by a particular protozoan is often referred to as being analogous to stages of cell differentiation in higher organisms. Certain ciliates also can use their locomotion organelles as sensory organs of touch.	Wet mounts or hanging-drop preparations may be used to show that protozoans are able to move by various mechanisms and organelles such as "amoeboid motion," cilia, or whiplike flagella.
5.31	Organelles	Protozoans contain discrete membrane-bound organelles.	Members within one group of protozoa (the Ciliata) possess two nuclei: a macronucleus that regulates cellular functions and a micronucleus that is associated with sexual reproduction. Macronuclei (sometimes more than one per cell) contain many copies of deoxyribonucleic acid (DNA), but do not function in mitosis or meiosis.	Transmission micrographs may be used to show organelles such as nuclei, mitochondria, etc.

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			Certain protozoans exist in diploid (2N) states during a major portion of their life cycle.	
5.4	Algae	Algae are photoautotrophic organisms that have various kinds of photosynthetic pigments.	The photopigment complement present in the chloroplasts of different algae determines the specific wavelength of solar radiation that can be used in photosynthesis. The biochemical reactions that occur during algal photosynthesis are similar to the kinds of reactions characteristic of plant photosynthesis.	Examination of living or preserved specimens can be used to show the photosynthetic organelles of algae.
5.41	Pigmentation	Chloroplasts are present in all algal cells. Pigments present in the chloroplasts of algae include chlorophyll *a*, *b*, *c*, and *d*. Other kinds of pigments vary considerably among species of different groups.	The colors of different kinds of algae result from the ratio of various chlorophylls and other pigments present in their chloroplasts. Bodies of water (ponds, lakes, streams, etc.) often appear greenish or red due to the abundant growth of algae. Such accumulations are called algal blooms.	
5.42	Cell Structures	These organisms have cell walls and various discrete membrane-bound organelles typical of eucaryotic cells.	Photoreceptors may be located in the membranes of chloroplasts. Chloroplasts in algal species may differ in shape, size, and the number present in cells. Chloroplasts are self-replicating organelles. They contain their own DNA, which differs from that found in the cell's nucleus. Cell walls of algae are composed of plant-like constituents, but certain kinds of carbohydrates are unique to particular groups. Rigid cell walls are conspicuously absent in organisms classified as euglenoids. Certain algal cell walls contain silica and polysaccharides (agar, alginates, and carrageenan).	
5.5	Viruses	All viruses are acellular, submicroscopic, obligate, intracellular parasites.	Viruses do not contain metabolic enzymes and ribosomes with which to carry out the process of protein synthesis.	
5.51	Nucleic Acid Components	Viruses contain either ribonucleic acid (RNA) or DNA, but never both.	Viruses are too small to be observed by light microscopy.	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	Spector, D. H., and D. Baltimore. 1975. The molecular biology of polio-viruses. Sci. Am. **232:**24–31.	Viruses in an extracellular state (virions) are infectious, but incapable of self-replication. The nucleic acid in the core of both DNA and RNA viruses may be either single- or double-stranded. **Fiddes, J. C.** 1977. The nucleotide sequence of a viral DNA. Sci. Am. **237:**54–67. The nucleic acid core of viruses is surrounded by a protein coat or other covering.	
6.0 Bacterial Structure and Function			
6.1 Cell Shape	Bacterial species exhibit one of three general forms: spherical (coccus), cylindrical (rod), or spiral.	Some bacteria do not exhibit one of the three general forms, e.g., some bacteria are stalked, some are branching, some are pleomorphic, and some exhibit characteristic extension of cell parts (prosthecate). If the cell wall of cocci, rods, or spirals is removed, they all tend to assume a spherical shape, e.g., spheroplast or protoplast.	Stained preparations of the three general bacterial shapes (coccus, rod, spiral) are easily observable with the ordinary microscope.
6.11 Cellular Arrangements	Depending on the species, bacteria exhibit a variety of arrangements of these shapes.	Some bacteria may display pleomorphism (irregular shapes). Some gram-positive and gram-negative bacteria may lack cell walls when grown in media containing an antibiotic such as penicillin, which blocks cell wall synthesis. Such cells usually display spherical shapes in high-salt environments and lyse in a low-salt environment, and some may not revert to walled organisms. When such wall-less organisms occur in nature, they are referred to as L-forms. Members of the family *Mycoplasmataceae* have no cell walls. Spiral-shaped bacteria such as vibrios may appear in the form of short, incomplete spirals. Others may appear as a thick, rigid spiral (spirillum) or a thin flexible spiral (spirochete).	Prepared microscope slides of cocci may be used to show morphological arrangements in pairs (diplococci), chains (streptococci), clusters (staphylococci), fours (tetrads), or cuboidal arrangements of eight (sarcinae).

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
6.2 Surface-Associated Structures	Several microorganisms have a variety of surface-associated structures. These include capsules, slimes, flagella, and pili.		
6.21 Capsules	Some bacteria are surrounded by a viscous layer termed a capsule or a slime layer which may serve a variety of functions.	Capsules are structures with a definite spherical shape surrounding the cell. The slime layer has no definite shape. Most capsules and slime layers are composed of complex polysaccharides. Some are composed of polymers of glutamic acid, e.g., *Bacillus anthracis*. Capsules may serve as extracellularly stored reserve food, may protect cells from dehydration and phagocytosis, are frequently related to virulence, and can be used in vaccines; some capsules are in commercially important products, e.g., the blood substitute dextran. The synthesis of capsules is greatly influenced by the bacterium's environment.	Staining techniques may be used to demonstrate bacterial capsules and/or slimes.
6.22 Flagella	Some bacteria produce hair-like appendages called flagella which serve as organelles of locomotion (Microbial Physiology, Subtopic 6.227).	Flagella originate in the cell membrane and are not visible with an ordinary microscope without the aid of special staining procedures. Flagella may be found in various arrangements. This property is used in bacterial classification. Bacteria displaying a single flagellum at one end of the cell are referred to as polar monotrichous; if there are more than one at one end, it is polarly lophotrichous; if there is one or more flagella at each end of the cell, it is amphitrichous; if flagella are inserted all around the cell, the arrangement is referred to as peritrichous. A few motile bacteria lack flagella and move instead by a gliding or flexing motion. Motile bacteria represent the simplest example of microbial behavior in nature in that they are capable of taxis (a motile response to an environmental stimulus) (Microbial Physiology, Subtopic 6.223). Flagella are composed of the protein flagellin. They appear as semirigid, noncon-	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		tractile, hollow fibrils and consist of a hook-like structure and a long filament. The ability to produce flagella is genetically determined. Flagella remain attached to the cell if the cell wall is removed.	
6.23 Pili	Certain bacteria possess filamentous submicroscopic appendages known as pili, which have several functions.	Pili have no specific arrangement. They generally protrude all around the cell, originating from the cell membrane, and are composed of the protein called pilin. One kind of pilus, the sex pilus, serves in the transfer of genetic material during conjugation (bacterial mating). It has been speculated that pilation is related to virulence, since pilated organisms are known to attach more efficiently to mammalian cell surfaces.	Electron micrographs may be used to show that not all bacteria possess pili, pili are thinner and more numerous than flagella, and piliated organisms tend to attach to other objects such as inert surfaces, living cell surfaces, and other bacteria.
6.3 Cell Wall	Most bacteria have rigid cell walls with a unique chemical composition. This chemical composition is frequently used in the classification of bacteria.	Bacterial cell walls are made of complex polymeric substances known as peptidoglycans, which are responsible for their rigid nature. The bacterial cell wall contains many different amino acids, amino sugars, carbohydrates, and lipids. Diaminopimelic acid (DAPA), muramic acid, and teichoic acid are unique to the bacterial cell wall. Peptidoglycans are very large polymers composed of three kinds of building blocks, acetylglucosamine (AGA), acetylmuramic acid (AMA), and a peptide consisting of four or five amino acids of a limited variety. Amino acids are of the D configuration, which is not usually found elsewhere in nature. Gram-positive bacteria have a cell wall composed almost entirely of a thick layer of peptidoglycan to prevent osmotic lysis. Teichoic acids are also components of such cell walls. Gram-negative bacteria have a multilayered cell wall consisting of a thin inner layer of peptidoglycan to prevent osmotic lysis surrounded by an outer envelope of lipopolysaccharide and protein (which may help to retain certain essential enzymes and pre-	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		vent some toxic substances from entering the bacterium). Gram-negative bacterial walls usually lack teichoic acid. The lipid component of the outer membrane of the gram-negative cell wall may also play a role in bacterial pathogenicity by acting as an endotoxin.	
6.4 Internal Structure	Internally, microorganisms may contain several structures including the cytoplasmic membrane, mesosomes, nuclear region, and ribosomes.		
6.41 Cytoplasmic Membrane	Immediately beneath the cell wall is a thin semipermeable membrane, the cytoplasmic membrane, that regulates the passage of materials into and out of the cell (Microbial Physiology, Topic 6).	The cytoplasmic membrane is semipermeable, composed of three layers, and houses a large number of enzymes including cytochromes and permeases. The bacterial cytoplasmic membrane, composed of proteins and lipids, is a typical biological membrane similar to those found in other cells. A cell may be killed by damage to the membrane from harsh treatment with physical or chemical agents. The cytoplasmic membrane invaginates to form various structures, i.e., mesosomes, photosynthetic lamellae, and vesicles, and frequently forms expanded tubules.	Electron micrographs may be used to show the cytoplasmic membrane.
6.42 Mesosomes	Mesosomes are internal extensions of the cytoplasmic membrane which are associated with a variety of cellular processes.	The mesosome system is intricately associated with bacterial nuclear material and its replication. Mesosomes are also involved with the beginning of cross wall formation.	Electron micrographs may be used to show that mesosomes may be lamellar membranes, expanded tubules, or vesicles.
6.43 Nuclear Region and Ribosomes	The cytoplasm is contained within the cytoplasmic membrane. Components found in the cytoplasm include the nuclear region, which contains DNA or the cell's genetic material, and ribosomes, which are involved with protein synthesis.	The cytoplasmic area is granular in appearance and rich in RNA. The nuclear area (nucleoid) is rich in DNA. The fluid contains dissolved nutrients. The nuclear region is not surrounded by a nuclear membrane and consists simply of a single, long, circular molecule of DNA (which codes for protein synthesis). High magnification with the electron microscope may reveal the nuclear substance to be a	Electron micrographs can be used to show that the cytoplasm contains all cellular structures and materials, including the nucleoid.

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		delicate fibrillar structure (bacterial chromosome). RNA in combination with protein forms submicroscopic bodies, ribosomes, which are usually densely packed throughout the cytoplasmic area. Aggregates of ribosomes are known as polysomes and are intimately associated with protein synthesis. Other inclusions are photosynthetic lamellae, vesicles, lipids, PHB granules (poly-B-hydroxybutyrate), glycogen, and metachromatic granules (reserve food material).	
6.5 Endospores	Certain gram-positive bacteria produce highly resistant and refractile thick-walled oval bodies termed endospores.	Endospores are surrounded by many laters of protective material, e.g., exosporium, spore coat, spore cortex, etc. These protective layers are apparently responsible for resistant to chemical and physical treatment. Endospores may germinate to become vegetative cells under suitable conditions. Heating endospores to near the boiling temperature for a few minutes may enhance germination (heat shock).	Laboratory experiments may be used to show that endospores are resistant to a variety of chemical and physical treatments.
6.51 Heat Resistance	The endospore wall contains a unique chemical known as dipicolinic acid (DPA) which is lacking in vegetative cells. A large amount of calcium is also present and is believed to form a calcium DPA-peptidoglycan complex.		
6.52 Sporeforming Organisms	Common sporeforming organisms include *Bacillus* (aerobic), *Clostridium*, (anaerobic), and various genera of the cyanobacteria.	Endospores of *Clostridium* species causing tetanus and gas gangrene may germinate in deep puncture wounds (anaerobic conditions) leading to disease production. The resulting vegetative bacteria produce the specific exotoxins responsible for the disease state.	
7.0 Viruses			
7.1 Characteristics	Viruses are submicroscopic (may not usually be seen with the light microscope).	The smallest virus which has been observed with the electron microscope is about	Electron micrographs may be used to show the submicroscopic, intracellular na-

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		0.01 μm in diameter (foot-and-mouth disease virus). Larger ones include vaccinia (cowpox) and variola (smallpox) (0.2 to 0.3 μm). Host cell nucleic acids and organelles are directed by the viral genome to synthesize new viral components. Such activity eventually may result in the death of the host cell. Depending on the virus and host cell system, virus particle release may be abrupt or gradual.	ture of viruses. The filterability of viruses can be demonstrated with the use of special filters.
7.11 The Virion	The mature transmissible virus particle is known as the virion.	Bacterial virions may be divided into two groups, lytic virions and temperate virions. Lytic bacterial virions generally destroy the host cell after the completion of the replication of new virions. Temperate bacterial viruses, on the other hand, do not replicate themselves but integrate into the host cell genome and remain integrated for an indefinite time.	
7.2 Virion Structure	Mature transmissible virus particles or virions consist of a protein coat, called a capsid, which surrounds the nucleic acid of the particle.	Most viruses have capsids. Proteins of the capsid confer specificity to the virus. Some viruses, such as bacterial viruses, shed the capsid outside of the cell after host penetration, whereas in other viruses the uncoating occurs within the host cell.	Electron micrographs may be used to show that the capsid is made up of protein subunits called capsomers and that certain virions, such as the herpesviruses, also may be covered by an additional component, the envelope or peplos, which may be derived from host cell membranes.
7.21 Nucleocapsid	The structure composed of nucleic acid surrounded by the capsid is the nucleocapsid.	General shapes of nucleocapsids include helical, polyhedral, filamentous, or binal, which is a combination of helical and polyhedral shapes. Certain nucleocapsids are quite complex. Some bacterial viruses, the bacteriophages, are composed of head, neck, collar, sheath, base plate spikes, core, tail fibers, and pins.	Electron micrographs may be used to show the variety of shapes, sizes, and organization of nucleocapsids.
7.22 Nucleic Acid Content	Individual virus particles contain DNA or RNA but never both. The nucleic acid component of virus particles is used as one criterion for classification.	The structure of nucleic acid in different viruses may be either linear or circular. Viruses are classified on the basis of whether they have double-stranded or single-stranded DNA or RNA.	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
7.23 Envelope	Some viruses have an irregular outer lipid-containing envelope, which encloses the viral capsid if present and the viral nucleic acid core. The presence of an envelope is one of several criteria used for classification.	Many mammalian viruses have an envelope (mantle). Such viruses are referred to as enveloped or mantle viruses. Envelopes appear to be derived from the host cell cytoplasmic membrane or nuclear membrane (depending on kind of virus and cell). Membranes contain lipids or lipoproteins and are composed of structural units sometimes called peplomers. Such viruses are sensitive to lipid solvents and are sometimes referred to as lipoviruses.	Electron micrographs may be used to show the properties of envelopes such as attachment spikes or projections and individual units known as peplomers.
7.3 Viral Replication	All viruses are obligate intracellular parasites.	All viruses have a certain host range (host specificity). Some viruses can penetrate and replicate in one type or kind of cell. Such viruses are referred to as monovalent viruses. If viruses are capable of infecting and replicating in two different types of cells, they are known as divalent, and if they penetrate and replicate in many kinds and types of cells, they are referred to as polyvalent viruses.	Laboratory experiments and electron micrographs may be used to show viral replication, e.g., plaque formation and pock formation in the chicken embryo.
7.31 Effects of Viral Replication	Certain viruses gaining entrance into susceptible cells may bring about the formation (replication) of new virus particles, which may or may not be accompanied by immediate cell death.	Cytopathic effects range from no observable physical change to complete cellular destruction. Virus replication differs with the type of virus and type of host cell involved. For example, one form of animal virus replication may start with adsorption, penetration, particle uncoating before penetration into the nucleus, DNA incorporation and transcription, RNA transport, synthesis of structural protein (capsid) and nonstructural protein (DNA synthetase), transport of structural protein and DNA synthetase into the nucleus, DNA synthesis, virus assembly inside the nucleus, envelope attachment as virus particles leave the nucleus (the nuclear membrane may become the envelope), and release. With certain viruses, cell lysis results in the concomitant release of new viral parti-	Laboratory experiments and micrographs may be used to show the range of destructive cellular effects caused by viruses, which are called cytopathic effects (CPE).

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		cles. With other viruses, viral particle maturation and release are relatively slow.	
7.32 Provirus	Viral DNA within the cytoplasm or in the nucleus may integrate into the host DNA and become a part of host DNA. Such a segment of DNA is referred to as a provirus. The presence of a provirus may change the host cell characteristics.	A provirus remains as part of the host DNA replicon indefinitely, or it may be spontaneously or intentionally induced to enter the lytic cycle. New virions derived from such a cell can invade a new host cell which does not contain the same virus (host cell immune response). However, a host cell containing another type of provirus may be infected, establishing double lysogeny. Invaded host cells may display cytopathic effects (CPE) and may produce chemical compounds which interfere with viral replication (interferon). Various physical and chemical agents may induce viral replication and consequent CPE. The exposure of seemingly uninfected cells to irradiation may bring about the activation of previously undetected viruses.	
8.0 Fungi			
8.1 Characteristics	Fungi include molds and yeasts.	Some molds produce useful antibiotics; others are parasitic and pathogenic. Certain molds under various environmental conditions exhibit different characteristic shapes (dimorphism). Most yeasts can grow aerobically, or anaerobically, or both. **Hartwell, L. H.** 1974. *Saccharomyces cerevisiae* cell cycle. Bacteriol. Rev. **38**:164–198. Certain yeasts may be pathogenic. Yeasts are used industrially for several processes including the manufacture of oils and fats, feed supplements, and alcohol and vitamin production.	Laboratory cultures and experiments may be used to show that molds are filamentous or thread-like, achlorophyllous mycelium-producing microorganisms and that reproduction can be asexual and/or sexual. Yeasts are primarily unicellular and generally reproduce asexually by budding. There are filamentous yeasts.
8.11 Multiple Forms	The fungi include unicellular and multicellular forms.	Several fungi produce large fruiting structures, e.g., mushrooms, bracket fungi, etc. Some fruiting structures are as large as 50 cm in diameter (puffballs), and are made of a mass of mycelia.	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			Some fungi are edible, whereas others are extremely poisonous.	
8.12	Hyphae	The structural units of most mature molds are the hyphae which may or may not have cross walls (septated or nonseptated).	Most members of the class Phycomycetes (bread molds, water molds, etc.) lack cross walls. Filamentous fungi in the classes Ascomycetes and Basidiomycetes are septate. The members of these classes include some unicellular forms (yeasts) as well as molds.	Microscopic examination of laboratory cultures may be used to demonstrate the presence or absence of septa.
8.2	Reproduction	All fungi except those of the class Deuteromycetes (Fungi Imperfecti) can multiply both asexually and sexually. Members of this group lack sexual fruiting bodies. The reproductive pattern of the fungi form the basis of their classification.	Asexual reproduction may involve binary fission, budding, spore formation, and the fragmentation of vegetative hyphae. Yeast form buds on the surface of the main or mother cell. Upon maturity buds are called daughter cells and have genetic constituents identical to the mother cell. Buds break off from the mother cell at maturity. Under suitable conditions, the mother cell can reproduce as many as 18 daughter cells by budding. In asexual reproduction spores may be zoospores, which become vegetative cells. Many fungi produce nonmotile vegetative spores. Spores may be borne on the tip of modified hyphae (sterigma) or enclosed in a sac (sporangium). Fungi can carry out meiois (chromosome reduction division, 2N → 1N). In some groups sexual reproduction occurs by the fusion of fertile hyphae from + and − strains of the organisms. Zygospores (2N) or zygotes (2N) result. Some sexual reproduction occurs by the fusion of two isogametes (gamete morphology is indistinguishable, e.g., slime mold) or by heterogametes (zoospore and egg). Sexual spores may be enclosed in a structure. The type of enclosure is used in the differentiation of fungi.	Asexual and sexual reproduction may be demonstrated by growing a "+" and a "−" strain of *Rhizopus* on the same plate.
8.3	Nutrition and Environments	The fungi are heterotrophic.	Fungi are extremely versatile organisms and are able to decompose a variety of com-	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			plex materials, thereby functioning as indispensable decomposers in our ecosystems. **Bauchop, T.** 1979. Rumen anaerobic fungi. Appl. Environ. Microbiol. 38:148–158.	
8.31	Growth Environments	Most fungi are aerobic, osmophilic, and acidophilic.	Fungi use molecular oxygen as a final electron acceptor in their respiration. Fungi are important in the food industry because of spoilage effects with syrup, molasses (high-sugar products), sauerkraut, oranges, and lemons. Fungi rarely spoil canned goods because of the anaerobic nature of such processed foods, but they are capable of forming toxic products in stored grains. **Maggon, K. K., S. K. Gupta, and T. A. Venkitasubramanian.** 1977. Biosynthesis of aflatoxins. Bacteriol. Rev. 41:822–855.	Laboratory experiments may be used to show that many fungi can tolerate or prefer a high-sugar or high-salt environment.
9.0	**Algae**			
9.1	Characteristics	The algae are photosynthetic unicellular or multicellular organisms. Some are filamentous or appear in colonial arrangements. Pigments and morphology are used in algal classification.	All algae contain discrete chloroplasts which can contain chlorophyll *a* and some other chlorophylls (*b*, or *c*, or *d*, etc.). Many algae possess accessory pigments, e.g., carotenoid (yellow-orange), fucoxanthin (brown), and phycoerythrin (red).	Laboratory experiments may be used to demonstrate algal motility.
		Multicellular algae exhibit a variety of shapes, ranging from single filaments to dichotomously branching large structures.	Algae share many properties with green plants; these include cell walls containing cellulose, chlorophylls *a* and *b*, and other pigments confined to chloroplasts and the production of starch in photosynthesis.	Examination of field or preserved specimens may be used to show that many algae, especially certain marine species, are not only multicellular but morphologically differentiated into holdfasts, stipes, and cysts.
9.2	Reproduction	Algae reproduce by both asexual and sexual means.	Unicellular algae can reproduce asexually by fission. Multicellular algae may reproduce asexually by zoospores or fragmentation. Sexual reproduction may be by fusion of isogametes. Multicellular algae may be heterogamous (eggs and sperm). Certain green algae (*Chlamydomonas*) can reproduce by fusion of two zoospores to produce a 2N zygote. Some multicellular algae, e.g., aggregates of similar cells, can reproduce sexually by	Examination of prepared microscope slides may be used to show that most unicellular algae reproduce by transverse binary fission; some reproduce by longitudinal fission.

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	Some algae exhibit alternation of generations in their life cycle. Such algae exist in both haploid (1N) and diploid (2N) states.	conjugation. Zygospores are produced upon fusion of two gametes in recipient cells. In most algae, the conspicuous (visible) generation is the vegetative structure (1N). Some zygotes may develop thick walls (cysts) to become metabolically dormant cells. Cysts may germinate when the environment becomes favorable.	
9.3 Physiology and Cultivation	Algae are photoautotrophs (Microbial Physiology, Subtopics 5.4 and 5.51).	Photosynthesis requires light as an energy source, chlorophylls, water, and carbon dioxide. The end products formed are sugar and free oxygen. Sugar may be polymerized and stored as polysaccharides, e.g., starch. Some algae may store lipids. Chloroplasts, like mitochondria, are capable of self-replication because they contain functional DNA and RNA. Some algae possess structures known as pyrenoids which may be involved in polymerization of glucose to starch. Most media for algal cultivation are composed of inorganic compounds containing bulk elements (C, N, P, S) and some trace elements including Fe, Mg, Zn, Cl, K, Mo, and B in addition to water. Algae can be grown on solid or liquid media.	Laboratory experiments may be used to show that algae are phototrophic and most do not require organic compounds in media.
10.0 Protozoa 10.1 Characteristics	The protozoans are unicellular, nonphotosynthetic eucaryotes.	The cytoplasm of most unicellular protozoans is divided into two regions: the inner fluid zone (endoplasm) and the outer or peripheral zone (ectoplasm). Some protozoans have an external covering known as a pellicle which may be composed of lipoprotein.	Examination of living and/or preserved specimens may be used to show that all protozoans lack rigid cell walls, some contain pellicles or periplasts equivalent to a cell wall, and others have a cell membrane as the outermost structure.
10.11 Means of Motility	Protozoans use a variety of means for locomotion which serve as a basis for their classification. The protozoans can be separated on the basis of motility into four groups: Sarcodina (pseudopodia), Ciliata (cilia), Mastigophora (flagella), and Sporozoa (some movement by pseudopodia only	Most protozoans are motile during at least one stage in their life cycles. Some protozoans move by means of flagellar motion, whereas others move by means of the sweeping motion of cilia. Both flagella and cilia consist of complex inner fibrillar structures not seen in procaryotic flagella.	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		in immature stages, male gametes are flagellated).	Flagella and cilia appear to originate in basal granules in the ectoplasm.	
10.2	Reproduction	Protozoans reproduce by both asexual and sexual means. The methods of reproduction and associated structures are classification criteria.	Protozoans can multiply asexually by binary fission. Budding may produce individuals exogenously or endogenously. Multiple fission occurs from the formation of multinucleate organisms. Subsequent cleavage results in the formation of many uninucleate individuals. Genetic recombination can result from the partial union of two cells (conjugation).	Examination of living or prepared microscope slides may be used to show asexual reproduction, which includes binary fission, multiple fission, budding, and plasmotomy. Sexual reproduction can occur through conjugation, syngamy, and autogamy.
10.21	Macro- and Micronuclei	Some protozoans are multinucleated.	Ciliates contain both macro- and micronuclei. Macronuclei play a role in metabolism and development. Those cells with only macronuclei are unable to conjugate. Cells with micronuclei can conjugate. Diploid micronuclei undergo meiosis and mitosis, resulting in two haploid nuclei (conjugal pair). One of these is exchanged during conjugation, forming a new diploid nucleus, which undergoes a complex series of nuclear divisions.	Examination of living cultures, prepared microscope slides, or electron micrographs may be used to show that ciliates have at least one macronucleus.
10.3	Life Cycles	Protozoans can be free-living or parasitic.	With a few exceptions, most members of Sarcodina, Ciliata, and Mastigophora are free-living, saprophytic scavengers in their respective habitats. All members of the Sporozoa are parasitic. Many protozoans have stages, namely trophozoite (active feeding stage) and cyst. Cysts are surrounded by thick walls and are dormant. Encystment occurs in an unfavorable environment, and excystment occurs when the environment becomes favorable. Many protozoan parasites have two different types of hosts: definitive, or final, and intermediate. The latter provides the environment for the immature or larval stage. Some protozoans produce serious and disabling diseases (malaria, African sleeping sickness, amoebic dysentery, vaginitis, gas-	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			tro-intestinal disturbances, and encephalomeningitis [*Naegleria* infection]). **Hawking, F.** 1970. The clock of the malarial parasite. Sci. Am. **222:**123–131.	
10.4	Nutrition and Cultivation	All protozoans are heterotrophs. Some ingest particulate material (holozoic); others absorb dissolved nutrients.	In some species (ciliates), food particles may pass through the peristome (oral groove) to the cytostome (mouth) and then into the cytopharynx (gullet). Ingested solid food particles are enclosed in food vacuoles. Wastes are eliminated via the cytopyge (anal pore). Other protozoa ingest food by phagocytosis or pinocytosis.	
10.41	Techniques	A variety of techniques and media are utilized for protozoan cultivation. **Dyer, C. L., and G. L. Butler.** 1980. A culture method for freshwater microinvertebrates. Am. Biol. Teach. **42:**52.	Nutrients used are similar to those for culturing of procaryotes. Sources for organic particles can be living (algae, bacteria, and other protozoans) or non-living (crushed or ground matter such as rice, wheat, etc.).	
11.0	**Microbial Ecology**			
11.1	Concepts of Ecology	Microorganisms are ubiquitous in nature and are often associated with the other living forms in their natural habitats. The major natural habitats within the biosphere are aquatic, terrestrial, and biological (in or on living systems) (Introductory Microbiology, Topic 25).	Subdivisions of habitats are called microenvironments. Microenvironments serve as integral parts of all habitats in ecosystems. **Imshenetsky, A. A., S. V. Lysenko, and G. A. Kazakov.** 1978. Upper boundary of the biosphere. Appl. Environ. Microbiol. **35:**1–5. Within microenvironments, microorganisms occupy specific niches. The niche of an organism is determined by genetic and physiological adaptation of the organism to either fixed or variable environments. Examples of adaptations to microenvironments include organisms that are halophilic (well adapted to a high-salt environment) and osmophilic (adapted to a high-sugar environment). Some organisms are psychrophiles (adapted to cold temperatures), some are mesophiles (adapted to moderate tempera-	Laboratory culture of environmental samples may be used to show the major habitats of microorganisms within the biosphere.

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		tures), and others may be thermophiles (adapted to high temperatures). Certain organisms preferentially thrive in acidic environments, whereas some thrive in neutral environments and others thrive in alkaline environments.	
11.11 Components of Ecosystems	Microorganisms comprise an integral part of the biotic community, which together with the abiotic (nonliving) components form ecosystems.	Biotic components in ecosystems are diverse and range from the simplest (cells) to the most complex (community). Microorganisms that serve as primary producers (blue-green bacteria) represent the first step in the pyramid of food levels supporting all other living things. Microorganisms function as important decomposers. Abiotic components in ecosystems tend to function as selective factors by making ecosystems conducive for certain kinds of organisms and undesirable for others. The acid pH of fruits in the presence of air tends to be highly selective for *Acetobacter* species. The temperature of water in hot springs in Yellowstone National Park is highly selective for thermophilic bacteria, e.g., *Thermus aquaticus.*	
11.2 Symbiotic Relationships	Symbiosis refers to the living together or to the association of two dissimilar organisms with a certain degree of constancy. Symbiosis may be divided into two general types: ectosymbiosis, in which one organism lives on the external surface of another organism, and endosymbiosis, in which one organism lives inside the cells, tissues, or organs of another form of life.	Many fungi are ectosymbionts of plants. Although they grow around the root system with little penetration into the host tissue, the association is beneficial to both fungi and plants, i.e., increased mineral uptake by plants (mycorrhizal association). Certain bacteria, e.g., members of the genus *Rhizobium*, establish endosymbiosis with leguminous plants. The bacteria infect the roots of legumes and establish themselves intracellularly in the plant tissue, producing root nodules. The bacteria are capable of fixing gaseous nitrogen, and the fixed nitrogen is provided to the plant. The association is beneficial to both plant and bacteria (mutualism). Endosymbionts (bacteria or algae) of cer-	Laboratory examination of field samples such as lichens and leguminous plants may be used to show symbiotic associations involving microorganisms.

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		tain *Paramecium* species are examples of symbiotic associations between two dissimilar kinds of microorganisms.	
11.21 Patterns of Association	Symbiotic relationships among living organisms can be categorized into three patterns of association. If an association of two or more dissimilar organisms or populations is beneficial to both, it is termed *mutualism*. If the association is beneficial to one without effecting the other, it is termed *commensalism*. If the association is harmful to one and beneficial to the other, the association is called *parasitism*. Among the various kinds of symbiotic associations that are found in nature, some do not require cell-to-cell contact between partners.	Endosymbionts (microorganisms) that live in the stomach of ruminants represent examples of a mutalistic association between unicellular organisms and higher animals. Lichens represent a unique biological entity that is formed from the mutualistic association between an alga and a fungus. Commensalism may be ectocommensalism (attachment of organisms to the host body) or endocommensalism (organisms which live in the lumen of the alimentary tract).	
11.22 Facultative Associations	A particular kind of symbiotic association may be facultative (nonobligatory) or obligatory.	Endomycorrhizae of orchids represent a highly specific symbiotic association between certain kinds of fungi and a flowering plant. Here the relationship is obligatory for the orchid and facultative for the fungi. Bacteria that are classified in certain genera (*Caedobacter*, *Lyticum*, and *Tectobacter*) are obligate endosymbionts of *Paramecium aurelia*. **Preer, J. R., Jr., L. B. Preer, and A. Jurand.** 1974. Kappa and other endosymbionts in *Paramecium aurelia*. Bacteriol. Rev. **38**:113–163.	
11.3 Microorganisms and Biogeochemical Cycles	Biogeochemical cycles are those processes through which chemical elements are transformed within ecosystems, often through the metabolic activities of microorganisms.	Molecular nitrogen (N_2) represents approximately 79% of atmospheric gases. The element nitrogen is an essential constituent for living cells (nitrogen cycle); microorganisms are responsible for all biological nitrogen fixation. Some microbes are capable of utilizing molecular nitrogen as a sole source of nitrogen (nitrogen fixers), whereas others are capable of oxidizing ammonia to nitrite or nitrate (nitrifiers) and others are able to	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		reduce nitrate to nitrogen (denitrifiers). *Nitrosomonas* species utilize ammonia as an energy source, and release nitrite as an end product. *Nitrobacter* species utilize nitrite as an energy source and release nitrate as an end product (Fig. 1). **Brill, W. J.** 1979. Nitrogen-fixation basic to applied. Am. Sci. **67**:458–466. Carbon is present in rocks and fossil material and is also an essential constituent of all living cells (carbon cycle) (Fig. 2). In environments where elemental sulfur is abundant and the pH is between 2 and 4, *Thiobacillus thiooxidans* oxidizes elemental sulfur. Sulfur may exist in various sulfates which may be reduced to H_2S by certain anaerobic bacteria. H_2S in turn may be oxidized to S or sulfate by lithotrophic bacteria (Fig. 3).	
11.31 Types of Biogeochemical Cycles	Geochemical cycles include carbon, nitrogen, phosphorous, and sulfur cycles. Certain microorganisms are highly specific in terms of a specific chemical structure (substrate) that they can metabolize. Chemical elements released to the environment as a result of microbial metabolism may selectively function as enrichment or inhibitory agents for other microorganisms. **Jannasch, H. W.** 1979. Microbial turnover of organic matter in the deep sea. BioScience **29**:228–232.		
12.0 Biological Molecules			
12.1 Proteins	Proteins are very large complex organic compounds composed of numerous small molecules called amino acids. They are important as enzymes and as structural elements of cells.	There are about 20 different amino acids. Hundreds or thousands of amino acid molecules combine to make proteins.	
12.2 Carbohydrates	Carbohydrates contain only carbon, hydrogen, and oxygen. They may be small molecules (monosaccharides) used as struc-	(Microbial Physiology, Subtopics 1.11, 1.21 and 1.33.)	

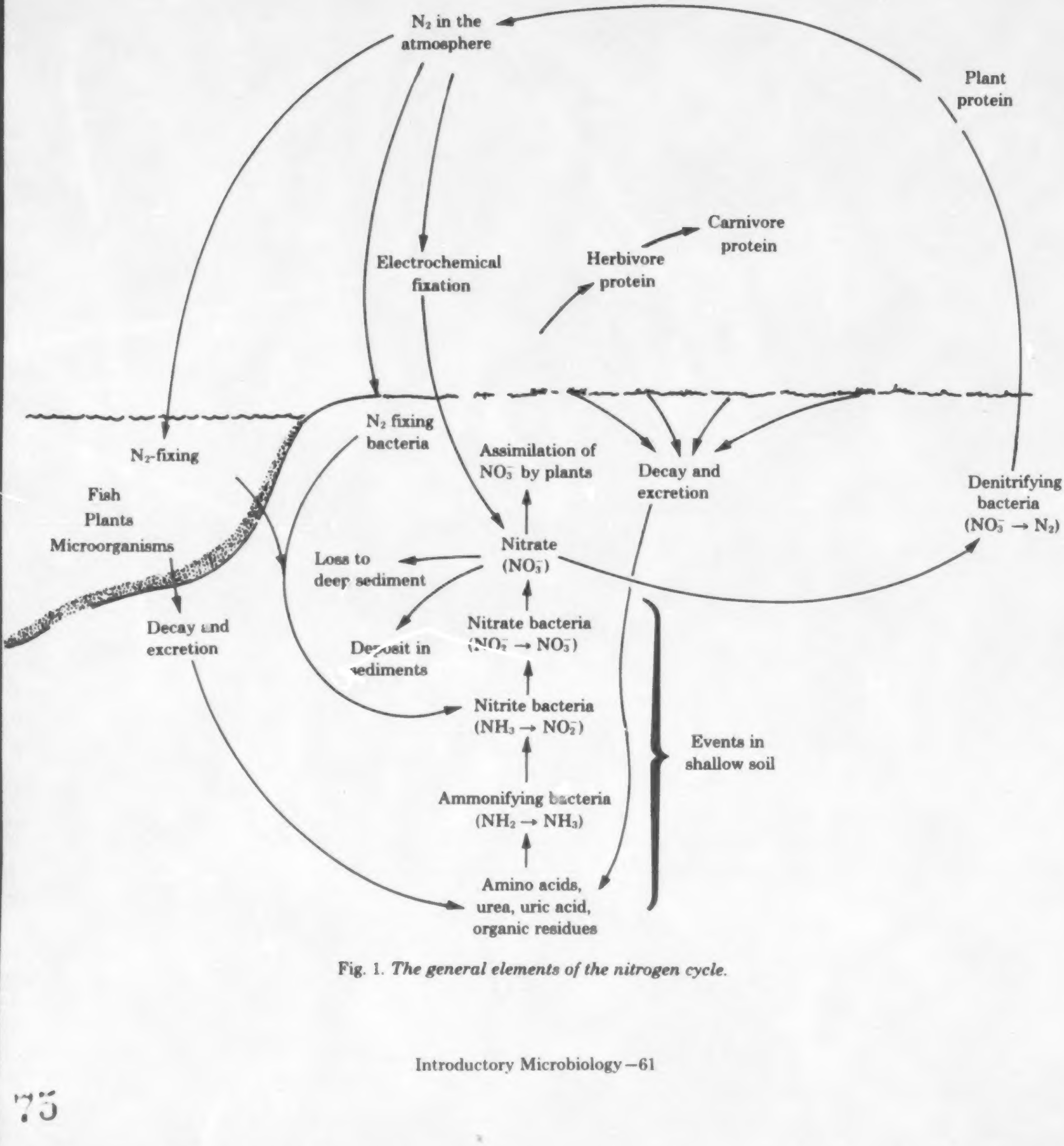

N_2 in the atmosphere
Plant protein
Carnivore protein
Herbivore protein
Electrochemical fixation
N_2 fixing bacteria
N_2-fixing
Fish
Plants
Microorganisms
Assimilation of NO_3^- by plants
Decay and excretion
Denitrifying bacteria ($NO_3^- \rightarrow N_2$)
Nitrate (NO_3^-)
Loss to deep sediment
Deposit in sediments
Nitrate bacteria ($NO_2^- \rightarrow NO_3^-$)
Decay and excretion
Nitrite bacteria ($NH_3 \rightarrow NO_2^-$)
Events in shallow soil
Ammonifying bacteria ($NH_2 \rightarrow NH_3$)
Amino acids, urea, uric acid, organic residues

Fig. 1. *The general elements of the nitrogen cycle.*

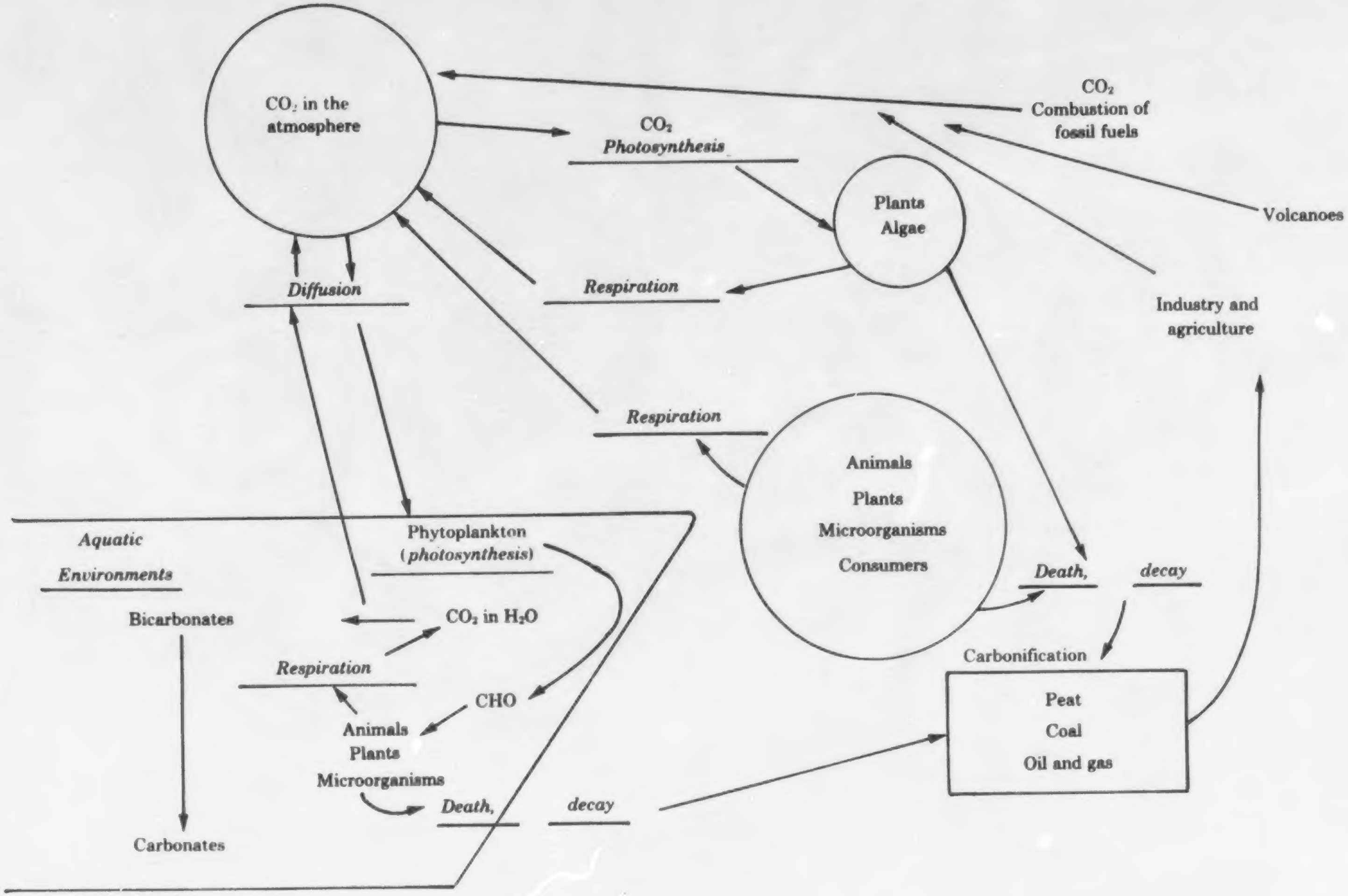

CO_2 in the atmosphere
CO_2
Photosynthesis
CO_2
Combustion of fossil fuels
Plants
Algae
Volcanoes
Diffusion
Respiration
Industry and agriculture
Respiration
Animals
Plants
Microorganisms
Consumers
Aquatic
Environments
Phytoplankton
(photosynthesis)
Death,
decay
Bicarbonates
CO_2 in H_2O
Carbonification
Respiration
CHO
Peat
Coal
Oil and gas
Animals
Plants
Microorganisms
Death,
decay
Carbonates

Fig. 2. *The general elements of the carbon cycle.*

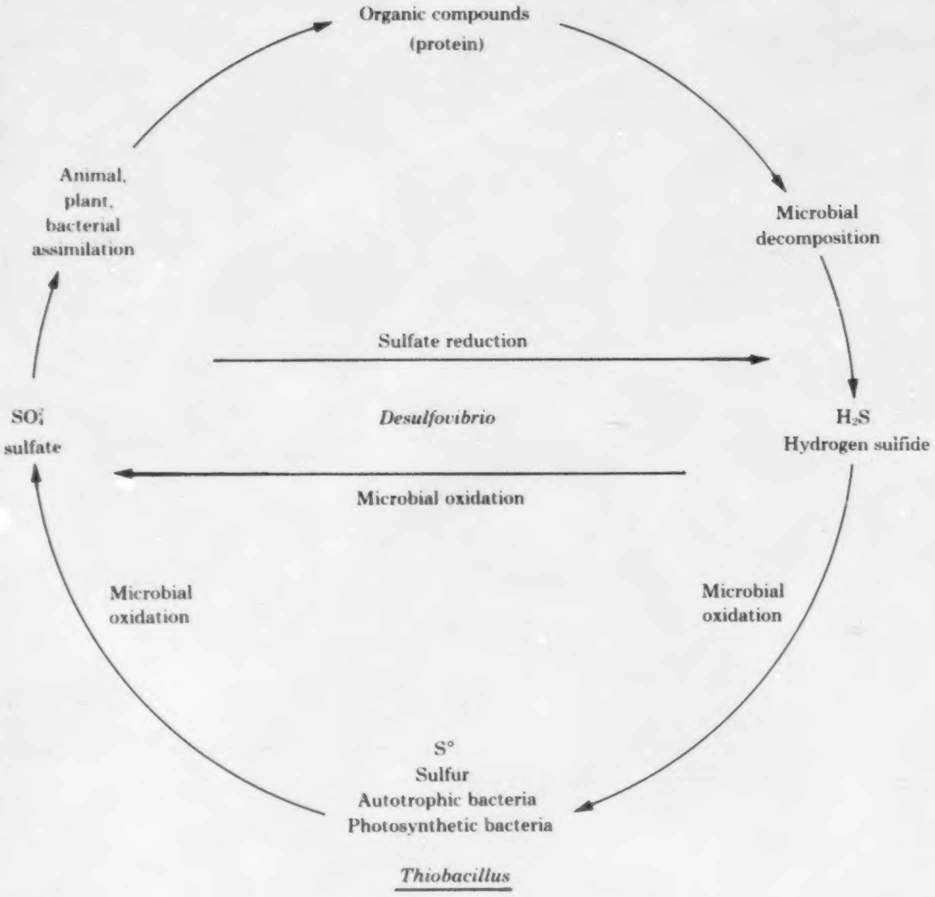

Organic compounds
(protein)
Microbial
decomposition
H_2S
Hydrogen sulfide
Microbial
oxidation
S°
Sulfur
Autotrophic bacteria
Photosynthetic bacteria
Thiobacillus
Microbial
oxidation
$SO_4^=$
sulfate
Animal,
plant,
bacterial
assimilation
Sulfate reduction
Desulfovibrio
Microbial oxidation

Fig. 3. *The sulfur cycle.*

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		tural elements of the cell or as a stored energy source.		
12.3	Lipids (Fats and Related Compounds)	Lipids are composed of carbon, hydrogen, and oxygen, but also may contain other elements such as phosphorus and nitrogen. They are insoluble in water and are important in intermediary metabolism and in structural elements such as the cell membrane.	(Microbial Physiology, Subtopics 1.13, 1.24, 1.31, and 1.34.)	
12.4	Nucleic Acids	Nucleic acids play major roles in the transmission of hereditary traits and the control of cell functions and protein synthesis.	(Microbial Physiology, Subtopics 1.14 and 1.23 and Microbial Genetics, Subtopics 2.1 and 2.2.)	
12.5	Cellular Composition	Microbial cells are composed of proteins, carbohydrates, nucleic acids, lipids, phosphates, and other materials. From 75 to 90% of the weight of the microbial cell is water.	Composition of bacterial cells will vary depending upon the kind of bacteria and the growth medium. General approximations of composition are as follows.	Wet weights and dry weights of a mass of concentrated bacterial cells can be determined to show the water content of the cell.

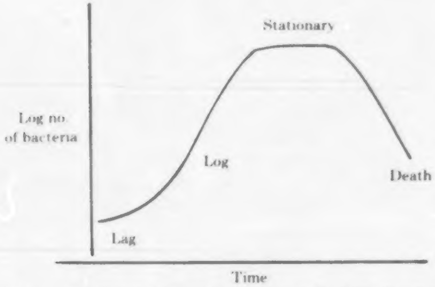
Component	% Dry wt
Water	75–90
Protein	35–50
Carbohydrate	2–25
Nucleic acids	
RNA	6–25
DNA	1–4
Peptidoglycan	
Gram-positive cells	15–20
Gram-negative cells	0.1–55
Lipopolysaccharide	
Gram-negative cells	5
Inorganics	
(phosphates)	0.9

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
13.0	**Microbial Growth**	Growth is the orderly increase in cellular constituents. Normal growth leads to cell reproduction. Growth of microorganisms leads to an increase in population.	In organisms that reproduce by binary fission, growth produces an increase in numbers. Coenocytic organisms increase only in individual cell size rather than in cell numbers. Single cells may continue to reproduce and form visible accumulations such as a colony (bacteria) and mycelium (fungus).	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
13.1 Measurement of Growth	It is possible to measure various aspects of microbial growth. One chooses the growth measurement technique needed depending on whether the emphasis is on total numbers, viable organisms, or metabolic rate of the population. Usually several techniques are used, and the results are compared.		
13.11 Increase in Mass	Measurements of increase in cell mass emphasize growth rather than reproduction. They may not distinguish living from dead cells.	Nephelometry and spectrophotometry are the most convenient and generally used growth measuring techniques for bacteria. The increase in turbidity of the culture is measured. Spectrophotometers measure the proportion of light which is transmitted through a filled cuvette placed in the light beam. Nephelometers (more sensitive) measure the proportion of light deflected under the same conditions.	A sample of washed cells may be dried in an oven, and the dry weight can be determined. A sample of washed cells may be analyzed to determine the total nitrogen or total protein. Cells in suspension scatter light; the amount of light scattered is proportional to the mass of cells per milliliter of fluid. A spectrophotometer or nephelometer can be used to measure scattered light.
13.12 Increase in Numbers	Cell numbers can be measured by direct microscopic counting. Culture techniques which test the ability of each organism present to grow to a visible mass or to metabolize and produce a detectable product are also used (viable counts). These methods are useful only for unicellular organisms.	Direct counts may be done to assist in standardizing other methods. They are also useful for quantitating unusual organisms in natural samples or hard-to-cultivate species. Automated electronic particle counters may be used. Viable counts are much used in studies of water samples, urinalysis, and food quality determination. A diluted sample is spread on a solid or mixed into a melted agar medium. If this is done with medium in a petri plate, it is called a plate count. Each viable organism may grow into a visible mass of cells (a colony). The number of colonies after incubation of this medium represents the number of viable bacteria in the diluted sample. Because it is difficult to know whether one bacterium gave rise to one colony, the number is usually given as colony-forming units (CFU).	The direct observation of a known volume of a specimen spread over a known area on slide can be used to calculate numbers of organisms per milliliter. The Petroff-Hauser counting chamber is used for direct counts of bacteria and other small cellular organisms. The count reflects both living and dead microorganisms. The plate count is used to determine the number of CFUs in a sample.

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
13.2 Factors Influencing Growth	Microorganisms are heterogeneous in their physical and chemical requirements. Optimum growth occurs only when all requirements are met.	When microorganisms are not growing because of inadequate conditions, they are not necessarily dead. Long periods of metabolic inactivity may be tolerated without a loss of viability.	
13.21 Nutrition	Growth patterns differ depending on whether nutrient supply is continuous (open system). Most natural situations are open; most laboratory conditions are closed. Microorganisms contain regulatory mechanisms that allow them to modulate growth for survival under changing nutrient availability.	See Microbial Physiology, Sub-Topic 3.30 for closed system information and Sub-Topic 3.4 for open system information. In natural situations such as bodies of water, organisms receive a continuous if fluctuating supply of nutrients. Wastes are also removed. Growth speeds up and slows down, but rarely stops. Under in vitro conditions, when nutrients are consumed, growth stops.	
13.22 Physical and Chemical Influences on Growth	Microbial growth is affected by various physical and chemical factors including the gaseous atmosphere, pH, temperature, and osmotic pressure.		
13.221 Gaseous Atmosphere	Microbial growth is affected by the concentrations of O_2, CO_2, and other gases in the surrounding medium. Most higher forms of life require O_2; many microorganisms do not. Obligate aerobes require O_2 for growth. Their metabolism is respiratory. Facultative anaerobes grow either with O_2 or without it. Growth is usually better in the presence of O_2. These organisms are usually fermentative in the absence of O_2. Obligate anaerobes do not grow in the presence of oxygen. Microaerophiles require reduced O_2 concentration and may require increased CO_2 concentration. Aerotolerant anaerobes are organisms living totally by fermentation in the presence of air. They are not sensitive to O_2.	Obligate anaerobes may be recovered from conditions in nature which appear to be aerobic. These organisms grow in microenvironments that are anaerobic. *Bacteroides,* an obligate anaerobe, can be isolated from the mouth. It inhabits anaerobic crevices around the teeth. Many anaerobes are killed by oxygen.	Aerobic and facultative anaerobes must have an O_2 source for best growth. In liquid culture best yields are obtained with small volumes of fluid in large vessels on shakers. Anaerobic growth conditions can be provided in sealed jars with catalytic oxygen removal or in anaerobic incubators. Thioglycollate, chopped meat medium, and media covered with mineral oil may be used to provide anaerobic conditions. A high-CO_2–low-O_2 atmosphere can be provided in a candle jar by combustion or in specially designed incubators. **Lennette, E. H., A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.).** 1980. Manual of clinical microbiology, 3rd ed., American Society for Microbiology, Washington, D.C.
13.222 pH	For most organisms the optimum pH for growth lies between 6.0 and 7.5. Some highly	Extreme pH occurs in some natural environments. Drainage from mining operations,	Detection of a pH change in media is usually done with pH indicators, e.g., phenol

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	specialized organisms can tolerate a pH as low as 1.0 or as high as 10.4. The internal pH of most microorganisms is close to neutrality regardless of the pH of the medium.	high mineral area, and certain fermentation conditions yield high-acidic environments. The inland salt or alkali lakes may have a pH as high as 11. Many important biochemical tests used in identification of bacteria depend on pH indicators to detect production of acidic or basic end products.	red is yellow below pH 6.8 and red above 6.8; methyl red is red below pH 4.5 and yellow above 4.5. Electronic pH meters can be used for exact readings in liquid media. Selective media for isolation of fungi usually have a pH below 6.0, whereas most general-purpose bacteriological media have a pH of 6.8 to 7.4.
13.223 Temperature	A given strain of microorganism will have an optimum temperature for growth. Growth will occur between its maximum and minimum temperature. These points are genetically determined. The effect of temperature on enzyme activity (Microbial Physiology, Subtopic 4.53) is important in determining the range over which an organism will grow. There are three groups of microorganisms with different ranges of growth temperatures: psychrophiles, mesophiles, and thermophiles. One commonly used definition of psychrophiles is that they have a growth range somewhere between −5° and 20°C. Mesophiles grow between 20 and 45°C. Thermophiles grow between 45 and 90°C.	Microorganisms grow over their entire growth temperature range. At suboptimal temperatures their generation time is extended from minutes to hours. Above the optimum, growth slows as the microorganism diverts ever-increasing amounts of cellular energy to repair heat-induced damage.	Soil or water samples may be plated and incubated at different temperatures to demonstrate presence of all three types of organisms. Many psychrophiles are killed by exposure to room temperature, so precautions must be taken to keep samples and media cold.
13.224 Osmotic Pressure	Osmotic pressure is determined by the concentration of dissolved particles in water. Microorganisms are found in environments of varying osmotic pressures. Most microorganisms, with the exception of protozoans, are protected from lysis in dilute environments by a rigid cell wall. Exposure to high osmotic pressure causes loss of water from cells. This arrests growth. The halophilic organisms live in highly saline environments (15 to 30% dissolved salts). They have a specialized membrane and salt-tolerant enzymes.	All types of dissolved colloidal particles contribute to the osmotic pressure of a solution. Cytoplasm has a relatively high osmotic pressure. The cell can vary this within limits by concentrating or excreting K^+. When the external environment contains more dissolved material than the cytoplasm, it is hypertonic, and the cell will tend to lose water to the environment. This drying effect arrests metabolism in most organisms; the effect explains the preservative value of salting or sugaring food. Dilute environments pose a challenge to bacterial survival. When the exterior environment is hypotonic, water tends to enter the cell. Expansion and death are prevented by the rigid cell wall. Cell wall-deficient forms of bacteria survive only	Mannitol salt agar may be used for the selective isolation of *Staphylococcus aureus* from human clinical samples because its high (7.5%) NaCl concentration inhibits almost all other nasopharyngeal organisms. The osmotic pressure of media for isolation of cell wall-deficient organisms, e.g., *Mycoplasma*, must be carefully adjusted.

	TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			in isotonic environments. Examples are the L-forms and mycoplasmas.	
13.3	Closed-System Growth	Within a closed system, the amounts of nutrients and their energy contents are fixed. Growth produces waste products which accumulate and may be toxic. (Fig. 4).  Fig. 4. *Phases of microbial growth in closed culture.*		All laboratory cultivation of microorganisms except continuous culture follows this model. The phases of growth may be demonstrated by inoculating fresh, prewarmed medium with an inoculum of log-phase cells and following the increase in medium turbidity. Any other accurate measurement of cell components may be used. By taking simultaneous samples for plate counts and direct counts, a good approximation of actual numbers may be obtained for each reading.
13.31	Lag Phase	Introduction of a microorganism that divides by binary fission into a sterile closed system such as a flask of nutrient broth starts growth. No appreciable increase in numbers occurs for a short time.	Each cell in the inoculum must carry out internal regulatory shifts to start growth. Accumulation of cytoplasmic components and increase in cell size begin. During lag phase, new proteins may need to be made. A complete round of DNA replication will precede the first and every subsequent cell division.	The duration of lag phase is extended if the inoculum is from an old culture, if the new medium is quite different in chemical composition from the old, or if it is at refrigerator temperature. During lag phase, measurement of cell constituents may increase.
13.32	Log Phase (Exponential Growth)	Once growth begins, increase in numbers is exponential for a period of time. The culture will commence division at the maximum rate possible given the genetic potential of the culture and the suitability of the medium.	During log-phase growth, readings should be made frequently because turbidity can double in as little as 20 to 30 min for some bacteria. Growth curve data may be plotted on linear graph paper with time as the abscissa and the log of the turbidity or the log of the number of organisms as the ordinate.	
13.321	Explanation of Exponential Growth	For most microorganisms one cell divides into two. Thus, during each generation time the population doubles. Starting with one bacterium you can illustrate exponential growth with the geometric progression: 1, 2, 4, 8, 16, 32 . . . or $2^0, 2^1, 2^2, 2^3, 2^4, 2^5 \ldots 2^n$.	All populations grow or decrease exponentially if each change reflects addition or subtraction of some constant percentage of the total individuals present at that time. If exponential growth proceeded for 48 h, one bacterium that divided every 20 min	During exponential growth there is a straight-line relationship between the log of cell number versus time.

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			would yield a bacterial mass weighing 4,000 times the weight of the earth.	
13.322	Generation Time	The generation time observed is the average time needed for one cell to complete a round of division into two cells. In this time the population in the closed system will double. The total amount of each cell constituent will double.	Generation times for some bacteria may be as short as 12 min. Thus, bacteria can multiply extremely rapidly under optimum conditions. Generation times for other bacteria and many eucaryotic microorganisms may be as long as several days.	Calculate generation time as follows: $G = \frac{t^1 - t^0}{n}$ or $\frac{t_1 - t_0}{3.3 \log_{10}{}^{b_1} - \log_{10}{}^{b_0}}$ t_0 = time at first measurement t_1 = time at second measurement b_0 = number of cells at t_0 b_1 = number of cells at t_1 G = doubling time or generation time n = number of generations
13.33	Stationary Phase	Environmental limitations put an end to exponential growth. The growth curve approaches a horizontal line. During the stationary phase some cells grow and divide. Some are active, others die, and the population number is unchanged.	Nutrient limitation is the major restriction for most aerobic organisms. Accumulation of toxic wastes is the most frequent cause of growth arrest in anaerobic culture.	The stationary phase may persist for a long period.
13.34	Death Phase	A decline in number of viable cells constitutes the death phase.	The death phase results in a reduction in viable cell numbers. This phase may or may not show a corresponding decline in total cell numbers.	The decline is usually exponential. Small numbers of viable cells may persist indefinitely at the end of the death phase.
13.341	Mechanism	An acute shortage of nutrients or high concentrations of toxic waste products may trigger activity of autolytic enzymes causing cell lysis.	The loss of viability without cell lysis can usually be attributed to an inability to supply the energy necessary to repair key genetic structures. When autolysis occurs, it is usually related to low cellular ATP levels, signaling activation of intracellular lysozyme. Clearing of the culture may be quite rapid if autolytic mechanisms are set in motion.	
13.4	Open-System Growth	Open-system growth occurs when constant environmental conditions are maintained. Nutrients are continuously provided, and wastes are removed. The number of cells per unit volume is kept constant.		

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13.41 Conditions	Laboratory devices for producing open-system growth provide a source of cells in the log phase of growth. They allow the study of a culture under optimum physiological conditions.	The growth rate of the bacteria in the vessel adjusts to the rate at which nutrients are provided. After a period of adjustment, the rate of increase of the cells through growth will just equal the rate of cell loss. The cells in a continuous culture apparatus are in log phase. The doubling time is determined by the growth conditions. If all else is constant, growth rate depends directly on the concentration of a limiting nutritional factor.	
13.42 Continuous Culture Devices	These are two types of continuous culture devices, chemostats and turbidostats. They are used for study of all activities of log-phase cells.	Continuous culture systems provide a convenient, constant source of log-phase cells for study. They are used for research on mechanisms of regulation, concentration of nutrients, selection of growth rate nutrients, and interactions among species in mixed cultures under conditions that simulate natural environments.	In a chemostat, the flow rate is set at a certain value. The rate of growth of the culture adjusts to the rate at which nutrients are added. In a turbidostat, an electronic device monitors turbidity, i.e., cell mass, of the fluid electronically signals the addition of fresh medium to maintain the desired population density.
14.0 Enzymes 14.1 Definitions			
14.11 Catalyst	A catalyst is a substance that affects the rate of a chemical reaction without being permanently altered itself.	Catalysts are used in a number of reactions of everyday practical interest and industrial importance. For example, platinum catalytically enhances the oxidation of unburned hydrocarbons in automotive exhausts (catalytic converters). Also, the industrial extraction of apple juice from apple pulp is aided by treatment with pectin-hydrolyzing enzymes (pectinases).	Starch + water → Glucose. This reaction will proceed at a very low rate in warm water. Upon the addition of a small quantity of amylase, accelerated glucose production (and starch hydrolysis) is noted. After a time, the enzyme can be isolated from the system with its original activity essentially undiminished.
14.12 Enzymes	Enzymes are highly specified catalysts produced by living cells. Enzymes have a protein component and may or may not have other components such as metal ions, vitamin, or carbohydrate molecules.		The fact that enzymes are protein can be demonstrated by their reaction with protein-detecting reagents such as Millon reagent or the Folin phenol reagent. Enzyme solutions will also exhibit maximum ultraviolet absorbance at 280 nm which is characteristic of protein solutions.
14.13 Substrate	A substrate is a substance which is altered in an enzyme-catalyzed reaction.		

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14.14	Active Site	An active site is a small area on the enzyme's surface which binds in a highly specific manner to the substrate. It is also called the catalytic site.	The active site can be pictured as a structure which matches up with a specific complementary conformation in the substrate in a "lock-and-key" sort of arrangement (Fig. 5).	
14.15	Allosteric Site	An allosteric site is a region on the enzyme surface, apart from the active site, where a regulatory substance may bind and affect the affinity of the enzyme for the substrate.	The allosteric site can be pictured as shown in Fig. 6. Note that the active site will "fit" the substrate but not the allosteric effector. The effector, if bound, results in a modification of the three-dimensional structure of the apoenzyme. This, in turn, modifies the structure of the active site.	
14.2	Mechanism of Enzyme Action	Enzymes work by lowering the required activation energy, thus facilitating the reaction (Microbial Physiology, Subtopic 4.3).		
14.3	Nomenclature	Enzymes are named according to the type of reaction catalyzed. They usually have the suffix -ase, e.g., gelatinase, lipase, etc.	The six basic classes of enzymes are oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases (Microbial Physiology, Subtopics 4.41 to 4.46).	
14.4	Factors Affecting Rate of Enzyme Reactions	The most important factors affecting the rate of enzyme reactions are enzyme concentration, substrate concentration, temperature, and pH (Microbial Physiology, Subtopic 4.6).		
14.5	Enzymes and Control of Microbial Metabolism	Since all biochemical reactions are catalyzed by specific enzymes, cells can control their function (metabolism) by changing the activity or amount of specific enzymes.	Only a small number of the possible enzymatic reactions occur in any cell at a given time. Some enzymes are only synthesized under particular conditions, and others are synthesized but inhibited from acting.	
14.51	Control of Enzyme Synthesis, Induction	Certain potential food molecules can trigger the microbial synthesis of enzymes necessary for their breakdown. This type of reaction is called enzyme induction.	*Escherichia coli* does not synthesize the enzymes for lactose metabolism unless lactose is present in its immediate environment (Microbial Genetics, Subtopic 6.12).	
14.52	Control of Enzyme Synthesis, Repression	The addition of a compound that is the end product of a biosynthetic pathway to a growth medium causes an arrest in the synthesis of the specific enzymes of the path-	End product repression is one of two feedback-regulating mechanisms for biosynthetic pathways. It regulates enzyme synthesis. End product repression is complemen-	

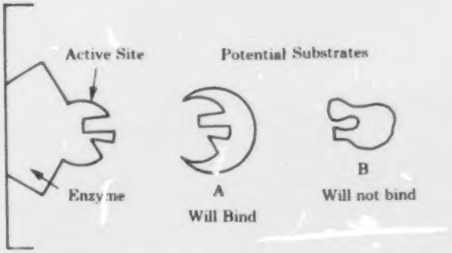
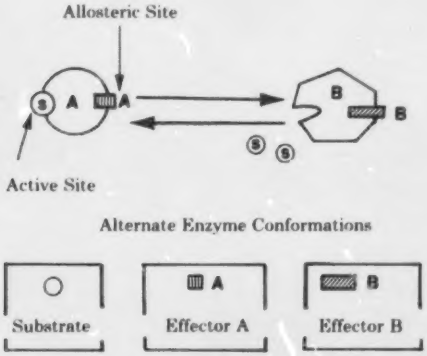

Active Site
Potential Substrates
Enzyme
A
Will Bind
B
Will not bind

Fig. 5. *Complementary structure of active site and substrate.*


Allosteric Site
S
A
A
B
B
S
S
Active Site
Alternate Enzyme Conformations
Substrate
A
Effector A
B
Effector B

If effector A is present, enzyme will be in a favorable conformation for interaction with the substrate. If effector B is present, substrate binding will be reduced because this effector stabilizes conformation B which cannot bind substrate.

Fig. 6. *The allosteric site and binding of positive and negative effectors.*

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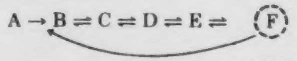
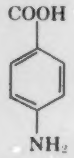
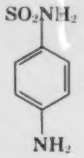
		way. This type of situation is called repression.	tary to end product inhibition (Introductory Microbiology, Subtopic 14.53). Both end product repression and end product inhibition serve to modulate the overall cellular metabolic activity to maximize the rate of growth through the efficient conversion of nutrients into cellular materials (Microbial Genetics, Subtopic 6.11).	
14.53	Control of Enzyme Activity	The activity of most enzymes is influenced by a variety of small molecules. Some of these socalled modulators stimulate enzyme activity, and some inhibit such reactions.	The relative concentrations of ATP, ADP, and AMP determine the ratio of catabolic to biosynthetic activity in a cell at a given time. ADP and AMP tend to stimulate catabolic enzymes; ATP tends to inhibit catabolic and stimulate biosynthetic enzymes.	
14.54	End Product Inhibition	When the end product of a metabolic pathway is present in excess, it may inhibit the action of one of the early enzymes in the pathway. This inhibition involves the allosteric site so the inhibitor does not compete with the substrate for the active site.	In the following reaction, an excess of an inhibitor (F) interacts with the allosteric site of the enzyme catalyzing the conversion of A to B. Since production of B is blocked, C will not be produced.  inhibitor	
14.55	Competitive Inhibition	Inhibitors which are structurally similar to the substrate compete with the substrate molecules for enzyme active sites. As the ratio of inhibitor to substrate is increased, a concomitant decrease in product formation occurs. Competitive inhibition can be reversed by increasing the ratio of substrate to inhibitor.	Consider the following structures:  (PABA) *para*-aminobenzoic acid  Sulfanilamide Some microorganisms utilize PABA as a substrate in the synthesis of folic acid, a material required for metabolism. The enzyme which normally binds PABA at its active site will also bind sulfanilamide. Therefore, if the ratio of sulfanilamide to PABA is high, the probability of the enzyme	

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		reacting with sulfanilamide rather than with its normal substrate is also high. The result is a decrease of folic acid synthesis and suppression of metabolism. Such inhibition is based upon the similarity in structure of normal substrate and inhibitor. Sulfanilamide does not effect the supply of folic acid to mammalian cells because they cannot convert PABA to folic acid and thus require folic acid preformed.	
15.0 Metabolism			
15.1 Cellular Chemical Reactions	Metabolism is the sum of all the chemical reactions in the cell.	In general, microbial metabolism is quite similar to the chemical reactions that occur in higher animals and plants. However, upon examination of individual microorganisms, there is a marked diversity of metabolic pathways and end products. This diversity is useful in identifying microorganisms such as bacteria and fungi. Many metabolic pathways known to be similar in all organisms were first worked out by investigators with microorganisms. *Escherichia coli* and *Saccharomyces cerevisiae* have been particularly useful in metabolic research.	
15.2 Energy	Energy is the ability to do work.	Microbial movement, growth, and reproduction occur only if there is an available energy source. The ultimate source of most biological energy is the sun. A possible exception to this is the deep sea volcanic gases system where the driving energy is the oxidation of H_2S by bacteria. **Ballard, R. D., and J. F. Grassle.** 1979. Return to oases of the deep. Natl. Geogr. **156:**689-705.	
15.21 Forms of Energy	Energy can exist as light or other kinds of radiation energy, chemical energy, mechanical energy, or heat.	The form of energy required directly for microbial movement, growth, and reproduction is chemical energy.	
15.22 Chemical Bond Energy	The energy of a molecule can roughly be represented as the sum of the forces holding its atoms together.		If a carbon-containing substance is burned (totally oxidized to CO_2), the heat produced is a measure of the potentially useful chemical energy of that substance.

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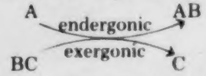
15.23	Endergonic and Exergonic Reactions	Certain bond formations between atoms require energy input (endergonic reaction); however, such energy can later be released by breaking the bonds (exergonic reaction).	A typical endergonic reaction may be represented as follows: $A + B + \text{Energy} \rightarrow AB$. A typical exergonic reaction may be represented as follows: $BC \rightarrow B + C + \text{Energy}$.	
15.24	Coupled Reactions	Biological transformations of chemical energy can occur because exergonic (energy-yielding) reactions are linked to endergonic (energy-requiring) reactions by common intermediates.	A coupled reaction is the sum of the following events: Endergonic: $A + B + \text{Energy} \rightarrow AB$ Exergonic: $BC \rightarrow B + C + \text{Energy}$ Sum: $A + BC \rightarrow AB + C$ Coupled reactions may also be written:  The reactions will proceed in the direction of the arrows as long as the energy released by the exergonic reaction exceeds that required by the endergonic reaction.	
15.25	Nucleoside Diphosphates and Triphosphates: ADP, GDP, UDP, CDP, ATP, GTP, UTP, CTP	These compounds are the most used intermediates between exergonic and endergonic reactions.	The conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP), for example, provides an efficient short-term energy storage and transfer mechanism in biological systems. The addition of a third phosphate to a nucleoside diphosphate is an endergonic reaction and can occur only when this reaction is linked to an exergonic reaction that releases sufficient energy. Enzyme-catalyzed removal of the third phosphate is exergonic; thus, it can be used to drive cellular energy-requiring reactions.	
15.3	Catabolism	Catabolism is the sum of cellular reactions resulting in the conversion of the energy from various organic molecules to energy usable for cellular work. ATP is the most "useful" cellular energy form available.		

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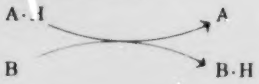
15.31	Oxidation-Reduction Reactions	Oxidation is removal of electrons and often protons (H^+) from a molecule. The electrons removed must be donated to another molecule. This recipient is said to be reduced. Thus, an oxidation reaction must always be accompanied by a reduction reaction. Oxidation-reduction reactions result in a transfer of energy. Much of the energy involved in the transfer may be lot to the system as neat, or if the reaction proceeds in a stepwise fashion, some of the energy may be conserved by coupled reactions.	In biological systems oxygen is one of the most avid available acceptors of electrons (most electronegative). Thus, an oxidation in which oxygen ends up with the transferred electrons releases the most energy.	An oxidation-reduction reaction can be represented as follows:  · = electron H = proton A = electron donor B = electron acceptor
15.32	NADs as Electron Carriers	Nicotinamide adenine dinucleotides (NAD^+) are the most common immediate electron acceptors of organic molecules. Reduced forms of these molecules (NADH and NADPH) act as electron donors in other reactions. NAD^+ is made from niacin and is a good example of the place of vitamins in metabolism.	The use of NAD^+ and $NADP^+$ as electron acceptors in catabolic processes is very important to the conservation of energy released by oxidation. NADH and NADPH do not donate electrons to oxygen directly and are relatively stable in the absence of enzymes specific for transfer of the electrons to a given substrate. This enables the organism to maintain control of its oxidation-reduction reactions.	For directions for performing a lactic dehydrogenase enzyme assay which will demonstrate use of NAD as an electron acceptor, consult: **Bergmeyer, H. U.** 1974 Methods of enzymatic analysis, vol. 1, p. 480. Academic Press, Inc., New York.
15.4	Degradative or Catabolic Pathways	Cells break down organic molecules by a series of enzyme-catalyzed reactions. A particular series of such reactions is called a metabolic pathway.		
15.41	Fermentation and Respiration	Fermentation and respiration are the two basic catabolic schemes. In fermentative pathways, the organic molecules being utilized as a source of energy are incompletely oxidized. No external inorganic electron acceptor is necessary (no O_2 is required). ATP is produced only by substrate level phosphorylation (Microbial Phyiology, Topic 5.25). The number of ATP molecules produced per molecule of substrate catabolized is small. In respiratory catabolism (sometimes called oxidative catabolism), the organic molecules being utilized are usually oxidized completely to CO_2. The electron transport	The type of catabolism associated with bacterial species is important to their characterization. Strict aerobes carry out respiratory catabolism only. Facultative anaerobes can utilize either fermentation or respiration. Aerotolerant anaerobes use fermentative pathways but are not harmed by O_2. Strict anaerobes use fermentative pathways and are harmed by O_2. A few are able to respire by using alternate electron acceptors (Microbial Physiology, Topic 5.2432). Two industrial processes with yeast can be used to demonstrate differences between fermentation and respiration. When yeast is used for alcohol production, the vats are	Simple laboratory tests can be used to determine whether a particular organism is obtaining energy from fermentation or respiration. One such test depends on the ability or inability of an isolate to utilize a particular organic energy source in a sealed tube. Information on oxidation-fermentation (OF) test media can be found in: **Lennette, E. H., A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.).** 1979. Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.

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		system (Microbial Physiology, Subtopic 5.24) serves to transfer electrons to an inorganic electron acceptor. Most of the ATP is produced by oxidative phosphorylation coupled to the electron transport system. The yield of ATP per molecule of growth substrate metabolized is much greater than the yield of ATP in fermentation.	kept strictly anaerobic. The ethanol is a product of fermentation. When yeast is produced for sale as bakers' yeast, it is grown under highly aerobic conditions, because the substrate added can only be obtained if the yeast is living by respiration.	
15.42	Glycolysis (Embden-Meyerhof Pathway)	Glycolysis is a pathway for the degradation of glucose found in most microorganisms. There are nine reactions needed to convert glucose to two pyruvate molecules. $\text{Glucose} + 2\,\text{NAD}^+ + 2\,\text{ADP} + 2\,\text{P}_i \longrightarrow 2\,\text{Pyruvate} + 2\,\text{NADH} + 2\,\text{ATP}$ There is one oxidative step in which NAD$^+$ accepts a pair of electrons from a three-carbon intermediate. The ATP molecules are produced by substrate level phosphorylation. The pyruvate and NADH generated have a different fate in fermentative organisms than in respiratory organisms.	Glycolysis is essentially the same in microorganisms, plants, and animals. It is a cytoplasmic cellular process, and the enzymes are not membrane-bound. The intermediate substrates are all phosphorylated. The pathway can be divided into three phases. The preparative phase converts glucose to two three-carbon phosphorylated molecules. This conversion requires two molecules of ATP. The oxidative phase converts the three-carbon molecules to the highly reactive 1,3-diphosphoglyceric acid (3-phosphoglyceroyl phosphate). This requires two molecules of NAD$^+$ and generates 2 NADH. The energy transfer phase results in substrate-level phosphorylation of 4 ADP to 4 ATP. Two reactions occur whereby energy and phosphate are transferred directly from the three-carbon intermediates to ADP (Fig. 7.).	
15.43	Other Pathways	Other routes exist by which sugars can be catabolized.	A unique characteristic of procaryotic cells is the diversity of catabolic pathways that may be found in different species.	The pathway by which glucose is catabolized may be an important taxonomic characteristic of some microorganisms. Most members of the genus *Pseudomonas* characteristically use the Entner-Doudoroff pathway.
15.44	Fermentation	Fermenting microorganisms are unable to carry out the reactions of electron transport either because they lack a terminal acceptor or because they lack the necessary enzymes	Because the energy yield per molecule of substrate utilized is low, a fermenting microorganism must catabolize more molecules of substrate than a respiring microorganism for	It is possible to identify certain taxonomic groups of bacteria by their fermentation products. The Voges-Proskauer test for the pres-

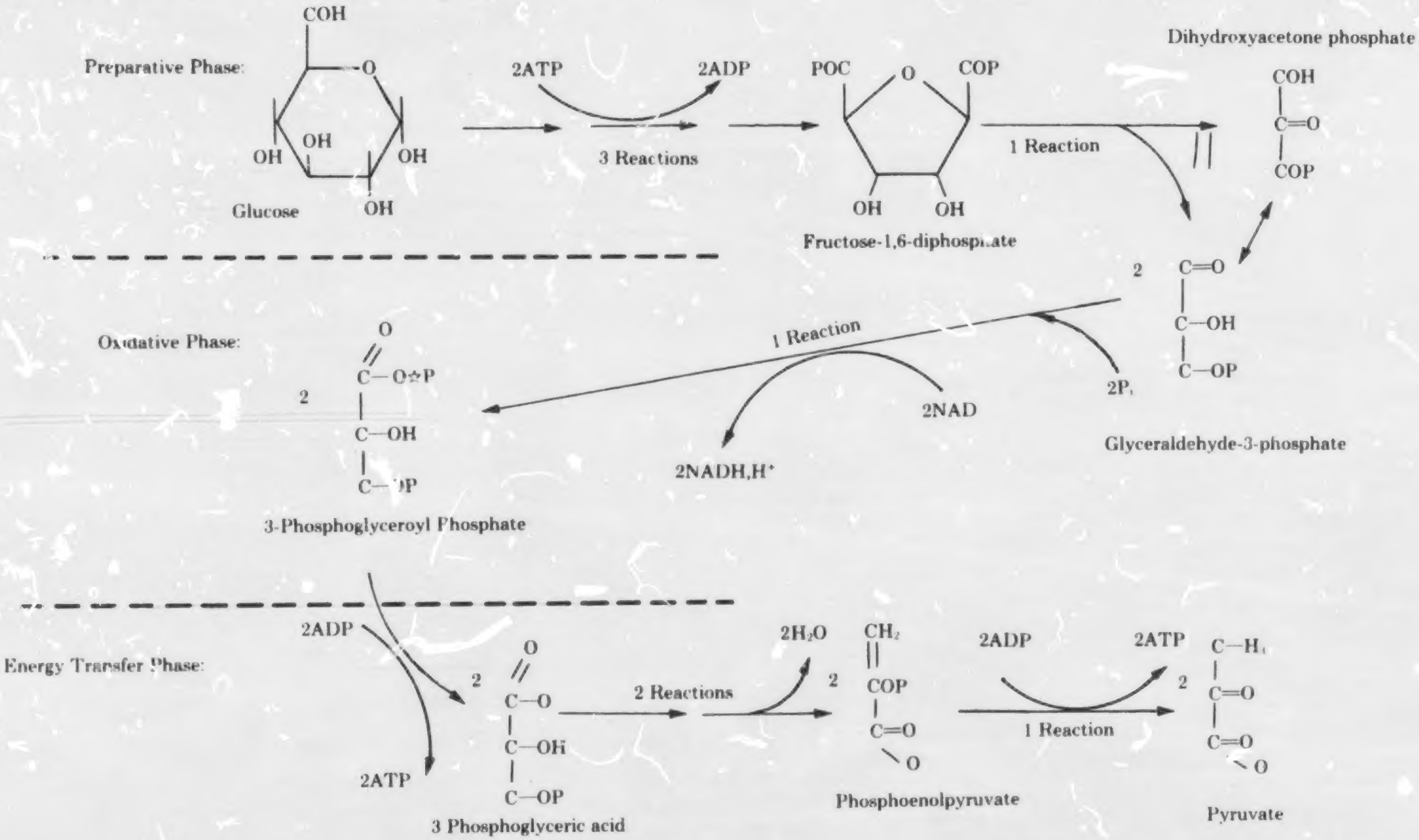
Preparative Phase:
COH
O
OH
OH
OH
OH
Glucose
2ATP
2ADP
3 Reactions
POC
O
COP
OH
OH
Fructose-1,6-diphosphate
1 Reaction
Dihydroxyacetone phosphate
COH
C=O
COP
2
C=O
C—OH
C—OP
2Pᵢ
Glyceraldehyde-3-phosphate
Oxidative Phase:
1 Reaction
2NAD
2NADH,H⁺
O
C—O☆P
2
C—OH
C—OP
3-Phosphoglyceroyl Phosphate
Energy Transfer Phase:
2ADP
2ATP
O
2
C—O
C—OH
C—OP
3 Phosphoglyceric acid
2 Reactions
2H₂O
CH₂
2
COP
C=O
O
Phosphoenolpyruvate
2ADP
2ATP
1 Reaction
C—H₃
2
C=O
C=O
O
Pyruvate

Fig. 7. *Glycolysis.*

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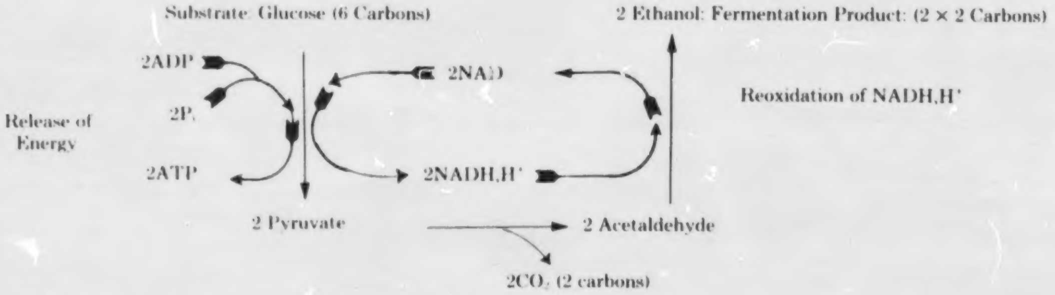
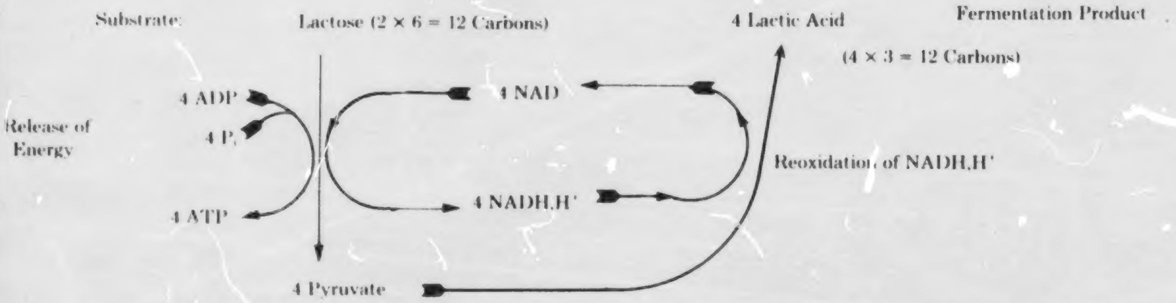
	or cofactors. They can grow and reproduce on the ATP produced by glycolysis or other anaerobic pathways, but because NAD^+ is in very limited supply they must be able to reoxidize the NADH produced to permit the anaerobic energy-yielding pathway to continue. Fermentative microorganisms utilize pyruvate or other products directly or indirectly as recipients for electrons from NADH. Thus, much of the pyruvate carbon can be found in the reduced organic molecules (fermentation products) excreted by fermenting organisms.	the same yield of growth and reproduction. In fermentations there is no exogenous electron acceptor, so the average oxidation level of the fermentation products must equal that of the substrates utilized. This requirement and the fact that no oxygen is available to carry out oxidative bond cleavage limit the range of substrates that can be fermented.	ence of acetoin is frequently used in identification of enteric bacteria. Two fermentative pathways are illustrated in Fig. 3.
15.45 Tricarboxylic Acid Cycle (Krebs' Cycle, Citric Acid Cycle)	Pyruvate is converted to acetyl coenzyme A (CoA) and CO_2. The further oxidation of acetyl CoA is dependent on enzymes of the tricarboxylic acid cycle. These enzymes are associated with the cytoplasmic membrane of procaryotic cells and are in the mitochondria of eucaryotic cells. For each acetyl CoA oxidized to CO_2, 3 NADH and 1 FADH are produced. These molecules donate electrons to the electron transport chain.	The tricarboxylic acid cycle has more than just a catabolic function. Many of the intermediates are starting material for synthesis of important cell components. Anaerobes that do not use the cycle for respiratory metabolism may still have many of the cycle enzymes. These have biosynthetic function. When tricarboxylic acid intermediates are removed for biosynthesis, the cycle can be kept in operation by synthesizing new oxaloacetate (Fig. 9).	
15.46 Electron Transport and Oxidative Phosphorylation	Electron transport is a cellular mechanism for conserving the energy released during oxidation of organic molecules. Catabolic processes involving participation of the electron transport chain are called respiration (Microbial Physiology, Topic 6.0).	The electron transport system consists of a series of molecules (flavoproteins, quinones, cytochromes) that are embedded in a cellular membrane. This series of molecules is referred to as the respiratory chain. These molecules have the ability to be both good electron acceptors and good electron donors. When NADH provides an electron pair, the electron transport molecules are alternately reduced and reoxidized in a particular order as the electrons pass from one to the next. As these stepwise oxidation-reduction reactions occur, energy is released, and this energy can be conserved by oxidative phosphorylation. For the continuation of electron flow through this pathway, there must be some exogenous compound that will	

The reduced organic molecules produced by fermenting microorganisms are of great commercial and diagnostic importance. Ethanol is produced mainly as a product of yeast fermentation.


Substrate: Glucose (6 Carbons)
2 Ethanol: Fermentation Product: (2 × 2 Carbons)
2ADP
2P_i
2ATP
Release of Energy
2NAD
2NADH,H⁺
Reoxidation of NADH,H⁺
2 Pyruvate
2 Acetaldehyde
2CO₂ (2 carbons)

Lactic acid, a product of the fermentation of milk sugar (lactose) by lactobacilli and streptococci, is important in production of cheese and yogurt.


Substrate:
Lactose (2 × 6 = 12 Carbons)
4 Lactic Acid
Fermentation Product
(4 × 3 = 12 Carbons)
4 ADP
4 P_i
4 ATP
Release of Energy
4 NAD
4 NADH,H⁺
Reoxidation of NADH,H⁺
4 Pyruvate

In the clinical laboratory, bacterial isolates may be identified on the basis of fermentation substrates and products.

Fig. 8. *Examples of fermentative pathways. In the clinical laboratory, bacterial isolates may be identified on the basis of fermentation substrates and products.*

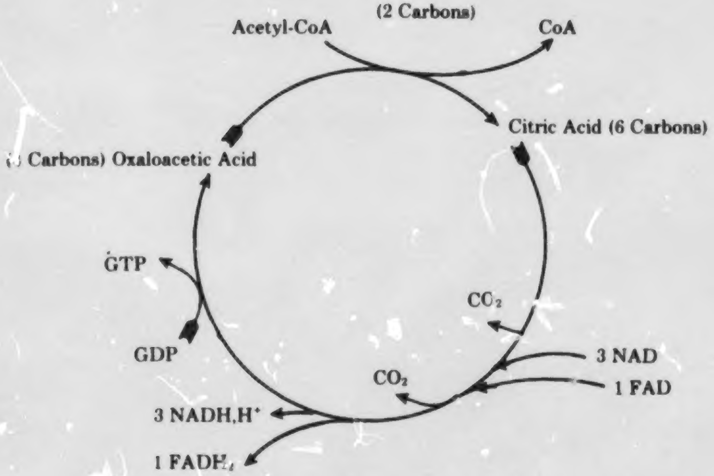

Acetyl-CoA
(2 Carbons)
CoA
Citric Acid (6 Carbons)
(4 Carbons) Oxaloacetic Acid
GTP
GDP
CO₂
3 NAD
1 FAD
CO₂
3 NADH,H⁺
1 FADH₂

Fig. 9. *A summary of the tricarboxylic acid cycle.*

<table>
<tr><th colspan="2">TOPICS AND SUBTOPICS</th><th>A. ESSENTIAL INFORMATION</th><th>B. ENRICHMENT INFORMATION</th><th>C. PRACTICAL ACTIVITIES</th></tr>
<tr><td></td><td></td><td></td><td>accept electrons from, and thus reoxidize, the last cytochrome in the chain.</td><td></td></tr>
<tr><td>15.47</td><td>Aerobic Respiration</td><td>Most microorganisms utilizing electron transport require oxygen to accept electrons from the final reduced cytochrome.</td><td>Obligate aerobes are microorganisms that cannot grow and reproduce in the absence of molecular oxygen. They require oxygen as a terminal electron acceptor.</td><td>Nitrate reduction can be observed in the laboratory by culturing denitrifying bacteria under anaerobic conditions with NO_3^- added to the medium. NO_2^- may be produced, e.g., *Escherichia coli*. Denitrifiers reduce NO_3^- to N_2 or other gaseous products, e.g., *Pseudomonas aeruginosa*.</td></tr>
<tr><td>15.48</td><td>Anaerobic Respiration</td><td>A few types of bacteria can use oxidized inorganic ions as electron acceptors in the absence of oxygen. NO_3^- (nitrate) and SO_4^{2-} (sulfate) are the most common alternate electron acceptors.</td><td>Bacteria that use NO_3^- as a terminal electron acceptor (denitrifiers) will use oxygen preferentially. The conversion of soil nitrate (NO_3^-) to gaseous nitrogen products by denitrifying bacteria is one way that soils lose nitrogen. The use of SO_4^{2-} as a terminal electron acceptor is restricted to a specialized group of anaerobic organisms. H_2S is produced. Water-logged sediments with an abundance of organic material and SO_4^{2-} (tideflats, for example) are noted for their H_2S-producing *Desulfovibrio* populations.</td><td>Winogradsky columns (Microbial Physiology, Subtopic 2.12) usually develop a good population of sulfate-reducing bacteria. It is possible to smell the H_2S produced in these cylinders or test for it with filter paper soaked in 5% lead acetate.</td></tr>
<tr><td>15.49</td><td>Pathways for Degradation of Other Substances</td><td>Microorganisms can use a wide range of carbon compounds as sources of carbon and energy. Series of degradation reactions, often catalyzed by inducible enzymes (Microbial Genetics, Subtopic 6.1), result in products that can enter the glycolytic or tricarboxylic acid cycle pathways.</td><td>Some synthetic molecules are very resistant to microbial degradation. DDT is one of these recalcitrant molecules.
<table>
<tr><th>Pesticide chemical</th><th>Approx. half-life (yr)</th></tr>
<tr><td>Chlordane</td><td>2–4</td></tr>
<tr><td>DDT</td><td>3–10</td></tr>
<tr><td>Dieldrin</td><td>1–7</td></tr>
<tr><td>Heptachlor</td><td>7–12</td></tr>
</table></td><td>Microorganisms vary greatly in their ability to degrade carbon compounds. Such substances need not be water soluble, e.g., crude oil. Microorganisms able to degrade large molecules often excrete the degradative enzymes into the medium containing the substrate. These excreted enzymes are called exoenzymes and can be detected by growing the microorganisms on solid medium containing the substrate and observing substrate disappearance around the areas of microbial growth.</td></tr>
<tr><td>15.5</td><td>Anabolism</td><td>Anabolism is the sum of the cellular chemical reactions that produce the organic molecules necessary for maintenance, growth, and reproduction of the organism.</td><td></td><td></td></tr>
<tr><td>15.51</td><td>CO_2 Fixation in Autotrophs</td><td>The conversion of CO_2 into the carbon compounds of cell material requires energy and reducing power.</td><td>The primary reaction in autotrophic CO_2 is the binding of CO_2 to the five-carbon sugar ribulose 1,5-diphosphate. This results in pro-</td><td>CO_2 fixation (the Calvin cycle) was first investigated in green algae. The path of incorporated ^{14}C-labeled CO_2 was followed in</td></tr>
</table>

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			duction of two phosphoglyceric acid molecules (each three carbons). These can be used as starting material to synthesize the carbon compounds needed by the autotroph.	photosynthesizing organisms. It was later discovered that this pathway is used by almost all autotrophs.
15.52	Relationship Between Anabolism and Catabolism	Catabolic metabolism provides sources of energy (ATP), reducing power (NADH and NADPH), and carbon skeletons for biosyntheses. Withdrawal of intermediates from catabolic pathways diminishes the net ATP yield. Control mechanisms function so that anabolic processes (biosynthetic pathways) are maximized when the ATP supply is high and catabolic processes predominate when ATP supply is low.		
16.0	**Introduction To Genetics**			
16.1	Definition of Genetics	Genetics is the study of the mechanisms by which the information of cells is stored, expressed, and modified and how this information is transmitted to other cells in the population and to future generations of organisms.	Generation times can be estimated by determining the increase in the number of colonies arising from samples of the growing population taken at various time intervals. For microorganisms reproducing by binary fission, the following formula can be applied: $G = T/3.3 \log (b/B)$	
16.11	Uses of Microorganisms	Microorganisms are useful tools in genetic studies for the following reasons: (i) microorganisms have short generation times; (ii) very large populations of almost identical cells can be produced asexually from a single cell in a short time; (iii) large populations can be grown in a small volume; (iv) microorganisms are usually haploid and gene expression is immediate; (v) and a variety of mutants can be isolated. **Riley, M., and A. Anilionis.** 1978. Evolution of the bacterial genome. Annu. Rev. Microbiol. **32**:519–560.	where G = generation time; T = time of cell growth; B = number of cells in original population; b = number of cells in final population. Since bacteria divide by binary fission, a single cell will give rise to over 1 million cells in 20 generations and to over 1 billion cells in 30 generations. Cell populations of bacteria approach 10 cells per ml in liquid cultures. Denser populations can be grown on the surface of solid media. Since microorganisms are haploid, a mutation will not be masked by its dominant counterpart. Genetic recombination in eucaryotic mi-	Inoculating "needles" may be used to transfer colonies in their exact position from an enriched medium to a variety of media lacking growth factors. Nutritionally defi-

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		croorganisms usually involves fusion of nuclei from two individuals to form a diploid nucleus. This is followed by meiosis. Haploid individuals develop characteristic of both parents.	cient mutants, auxotrophs, can be identified by their failure to grow on one or more deficient media.
17.0 **Genetically Important Macromolecules**			
17.1 Deoxyribonucleic Acid, Ribonucleic Acid, and Protein	The major molecules important in genetics are deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein. **Cohen, S.** 1975. The manipulation of genes. Sci. Am. July **233**:24–33.	The genetic information coded in DNA is transcribed into mRNA which is then translated into protein. This flow of information constitutes the central dogma of molecular genetics. A diagramatic form of this dogma would be DNA → RNA → protein. Messenger RNA, transfer RNA, and ribosomal RNA are involved in protein synthesis. In RNA viruses, reverse transcriptase is the enzyme which produces a DNA copy of the RNA genome of the virus.	
17.2 Deoxyribonucleic Acid (DNA)	DNA is a double-stranded polymer of nucleotides. Each nucleotide consists of a phosphate group, deoxyribose sugar, and one of four bases: adenine, guanine, thymine, or cytosine. The two strands are held together by hydrogen bonding between the complementary base pairs adenine and thymine and guanine and cytosine.	In procaryotes, DNA is a small circular molecule without a surrounding membrane. Procaryotes do not have histones. Eucaryotic DNA is divided into chromosomes. The DNA is combined with histone and nonhistone proteins to form a complex structure. The number of chromosomes varies among different eucaryotic organisms, and these chromosomes are located in the nucleus, a membrane-bounded structure. Mitochondrial and chloroplast DNA are small circular molecules located in these membraneous organelles.	The nuclear region of procaryotes can be demonstrated by the Feulgen staining procedure. Staining of eucaryotic cell nuclei or chromosomes is achieved by use of basic dyes.
17.21 Functions of DNA	DNA has two major functions: to replicate itself and to code for the synthesis of protein. A gene is the sequence of nucleotides which codes for a single protein. The entire complement of genes in an organism comprises its genome. **Mirsky, A.** 1968. The discovery of DNA. Sci. Am. June **218**:78–88.	In biosynthetic pathways which require numerous enzymatic steps, the genes coding for the various enzymes may be linked into a unit called the operon. An operator gene and a regulator gene control the operon's functioning.	One evidence of DNA as the genetic information carrier comes from the experiments of Hershey and Chase (1952), who demonstrated that only the DNA of the virus entered a bacterial cell.
17.22 Replication	The two DNA strands separate at a spe-	Replication is catalyzed by DNA polym-	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	cific point called the origin. Enzymes catalyze the synthesis of two new strands complementary to each of the original two strands. The two new DNA molecules formed are identical to the original molecule.	erases which require a single-stranded template; the mechanism by which the double-stranded molecule separates is unknown. DNA replicates itself in a semiconservative process as described by Meselson and Stahl in 1957. The two strands of the double-stranded DNA molecule are complementary in base sequence. Each strand serves as a template to which a new strand is formed, resulting in two DNA molecules having one strand from the original DNA molecule and one strand newly synthesized. The process also requires a primer segment of polyribonucleotide, 50 to 100 nucleotides long, produced by a DNA-dependent RNA polymerase (probably different from that involved in RNA synthesis). Replication is discontinuous and occurs in a 5′ to 3′ direction on both strands of the open region of DNA as demonstrated by Okazaki in 1968 (Fig. 10). The RNA-DNA fragments found in the initiation processes of replication are termed Okazaki fragments.	
17.3 Ribonucleic Acid (RNA)	RNA is a single-stranded polymer of nucleotides. The nucleotide subunit of RNA differs from that of DNA in that ribose replaces deoxyribose and uracil replaces thymine. There are three major types of RNA: messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). **Rich, A., and H. K. Sung.** 1978. The three-dimensional structure of transfer RNA. Sci. Am. January **238**:52–62.	The three types of RNA are coded in the specific base sequences on the DNA molecules. The maturation process of the three kinds of RNA differs and results in specific spatial configurations for each RNA type. rRNA constitutes up to 65% of the ribosomal mass. Procaryotic ribosomes have three characteristic forms of rRNA; eucaryotic ribosomes are larger and have four types of rRNA.	
17.31 Functions of RNA	The three types of RNA function at various stages in protein synthesis	mRNA is involved in both transcription and translation processes of protein synthesis. A specific mRNA is a transitory molecule. The tRNA is folded in a "cloverleaf" structure. Each of the 20 amino acids found in proteins has at least one corresponding tRNA. Some amino acids are carried by more than one tRNA. The mRNA codons	

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			and the corresponding amino acids in the genetic code are known.	
17.4	Protein Structure	A protein is a linear polymer of amino acids joined together by peptide bonds. The protein has a unique three-dimensional structure determined by its amino acid sequence.	Furthermore, secondary and tertiary configurations of a protein are determined by the primary structure. Proteins can associate noncovalently with other proteins in specific arrangements, resulting in a quaternary configuration.	
17.5	Protein Function	Proteins function as enzymes and as structural elements.	As enzymes, the protein has specific sites at which a substrate molecule is changed to the product of the enzyme's action. This site is called the active or catalytic site. As structural elements, proteins constitute at least 50 to 60% of the cell membrane.	
17.6	Protein Synthesis, Transcription	In transcription the mRNA molecule is synthesized complementary to a gene on one strand of DNA. The mRNA bases form the pattern to be translated into the specific amino acid sequence of a protein. A triplet of three nucleotides is called a codon and will be translated into one specific amino acid of the protein.	The DNA-dependent RNA polymerase binds to specific initiation sites on the DNA to begin transcription of a specific RNA. The initiation site-binding complex results in localized strand separation of the DNA and the beginning of transcription (Fig. 11).	
17.7	Protein Synthesis, Translation	In translation the mRNA molecule is decoded on the ribosome by the tRNA molecules carrying amino acids. Each tRNA attaches to a specific codon of the mRNA. The ribosome moves along the mRNA, resulting in the sequential polymerization of amino acids to form a protein (Fig. 11).	Translation of mRNA involves four major steps. (i) Activation of tRNA. This involves the binding of an amino acid to tRNA. (ii) Initiation of translation. The first initiating aminoacyl-tRNA binds to the small subunit of the ribosome with the involvement of specific proteins. The large ribosomal subunit attaches to this complex, thus forming a functional ribosome, ready for the next step. (iii) Combination of amino acids into an elongating protein chain. The polymerization of amino acids is accomplished by the sequential transfer of amino acids from the tRNA molecules which attach to the mRNA at the ribosomal positions, peptidyl site (P) and aminoacyl site (A). After formation of	

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		the peptide bond between the two amino acids attached to the P and A sites on the ribosome, the ribosome moves one codon further along the mRNA chain: the peptidyl site contains the growing polypeptide site, whereas the aminoacyl site accepts a new aminoacyl-tRNA which carries the anticodon corresponding to the new codon on the mRNA molecule. In this stepwise fashion, each succeeding codon is translated into an amino acid and eventually into the protein chain. (iv) Termination. The protein chain is completed when the appropriate termination codons are reached on the mRNA molecule. The protein product is released from the ribosomes.	
18.0 Microbial Genetics			
18.1 Procaryotic Chromosome	There is one closed circular chromosome DNA molecule per cell which attaches to the cell membrane at a specific attachment site. No structural proteins are associated with the DNA molecule.	The sequence of bases in the DNA may be used as a measure of the relationship of different species. The closer the sequence, the closer the relationship.	The bacterial chromosome may be visualized by autoradiography. The bacterial chromosome attached to the bacterial membrane may be isolated by detergent lysis of the cell followed by centrifugation.
18.2 Plasmid	A plasmid is an extrachromosomal, closed circular molecule of DNA which varies from 0.1 to 10% the size of the chromosome. Cells may contain multiple copies. The plasmid is not necessary for essential normal cellular activities. Some plasmids may integrate into the bacterial chromosome. **Clowes, R. C.** 1973. The molecule of infectious drug resistance. Sci. Am. April **228**: 18–27.	Plasmid replication may be under its own control or that of the chromosome. Plasmids must contain genes for their own replication and may contain genes for transfer and for the synthesis of other products. Plasmids can be divided into various classes depending on the products for which they code. These include drug resistance, bacteriocin, and toxin synthesis. Plasmids in an integrated state may mobilize transfer of a chromosome to another cell. A plasmid may carry a chromosome fragment. In a state of this type it can transfer bacterial genes.	Plasmids may be physically mapped by cleavage into fragments by enzymes (restriction enzymes) which cut at specific sites. Each plasmid yields a distinct and reproducible fragment pattern.
18.3 Eucaryotic Chromosome	The genetic material of eucaryotic microorganisms is located mainly in the form of	Eucaryotes contain several basic proteins (histones bound to their DNA). These help	The chromosome number may be determined in some cases by staining the chro-

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	multiple DNA molecules called chromosomes, which also contain bound structural and regulatory proteins. The cell can be haploid or diploid.	determine the chromosome structure. They also have acid proteins bound to the DNA which are involved in regulation of gene expression.	mosomes and counting and can often be estimated by linkage analysis.
18.4 Eucaryotic Organelles	Both mitochondria and chloroplasts contain small procaryote-like chromosomes. **Goodenough, U., and R. P. Levine.** 1970. The genetic activity of mitochondria and chloroplasts. Sci. Am. November. **223:** 22-29.	Organelle DNA contains genes for ribosomal RNA, ribosomal protein and some, but not all, transfer RNAs. Organelle DNA contains some genes for enzymes such as cytochrome oxidase in mitochondria.	Purified DNA from organelles is generally isolated by first purifying the organelle.
18.5 Procaryotic DNA Transfer	DNA is transferred from donor to recipient cells by three different mechanisms: transformation, conjugation, and transduction. Once inside the recipient cell, the donor DNA may recombine with the recipient chromosome. Plasmids also may be transferred by transformation, conjugation, and transduction.	Transfer is recognized if the donor DNA differs from the recipient DNA, and the mixture of cells is plated on a medium on which only recombinants will be able to grow and form colonies. Transfer has been recognized in only a small minority of all bacteria. However, this may be because the proper conditions have not been used to demonstrate transfer.	
18.51 Transformation	In DNA-mediated transformation, DNA fragments released from lysed cells are taken up by recipient cells.	Addition of deoxyribonuclease to the lysed donor cells prevents transfer by degrading the donor DNA. The recipient cells must be grown in a certain way to gain the ability to take up such a large molecule as DNA. Such recipient cells are termed competent.	
18.52 Conjugation	In conjugation the donor DNA is transferred during the time that the donor and recipient cells are held in contact, by a sex pilus of the donor (F^+) bacterium. **Wollman, E., and F. Jacob.** 1956. Sexuality in bacteria. Sci. Am. July. **195:**109-116.	Chromosome transfer is detected if the donor and recipient cells are plated on a medium on which neither will grow, but on which cells with a combination of the donor and recipient cells' properties will grow. Most cells attach to one another by means of pili. DNA is transferred only as long as the cells remain in contact.	
18.53 Transduction	In transduction the donor DNA is transferred from the donor to recipient cells by a bacteriophage (phage). **Zinder, N.** 1958. Transduction in bacteria. Sci. Am. November. **199:**38-43.	The bacterial DNA takes the place of phage DNA inside the protein coat of the phage; thus, the phage is "defective" since it does not have all of the genetic information required for its own replication.	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		The recipient cells are plated on a medium on which they will not grow unless they are provided with additional genetic information. Only recipient cells that are not lysed by the phage can be transduced.	
18.6 Mutation	A mutation is an inheritable change in the base sequence of DNA. A mutation may change any characteristic of a cell or a virus.	Mutations can be either spontaneous or induced. Mutations may or may not cause an observable change in phenotype depending upon the extent or change in an enzyme. Examples of types of mutation are as follows. Structural. Loss of ability to produce a capsule; loss of ability to produce flagella (nonmotile mutant); change in colonial morphology. Nutritional. Loss of ability to synthesize a biosynthetic enzyme, thereby producing a requirement for a particular nutrient or growth factor not required by the parent cell; parent is the prototroph and the nutritional mutant derived from it is called an auxotroph. A drug- or virus-resistant mutant can grow in the presence of a drug or virus which kills or inhibits the growth of the parent. Loss of degradative enzymes is manifested by loss of ability to use a given compound as a carbon source or energy source. Temperature-sensitive mutants grow at one temperature but not at another. This is an example of a conditional lethal mutant, that is, an organism that grows only under conditions not required for growth of parent.	Phenotypic expressions of mutations may be detected by direct observation of colony color, size, texture, or other colonial property; use of differential media; isolation of nutritional mutants by replica plating; and the use of penicillin selection (bacteria) or filtration technique (molds) to enrich for mutants. Exposure to ultraviolet (UV) light and X rays may increase the yield of mutant cells. Examples of mutants include pigmentation mutants as colonies on solid media; phage-resistant mutants identified as colonies forming on agar plates which have been sprayed with phage; antibiotic-resistant mutants isolated on media containing the antibiotic; and replica-plating techniques using a velveteen pad as an inoculating device to transfer colonies in their exact position from a plate containing an enriched medium to a variety of media lacking particular growth factors.
18.61 Base Sequence Changes	The base sequence of DNA may be changed by substitution of a base; addition or deletion of a single base; or deletion or addition of a segment of DNA.	Mutational events involving a change in a single base or base pair are called point mutations. Ultimately, all mutations occur because of change in the base pair sequence in DNA synthesis.	
18.62 Mutation Frequency	The rate or frequency with which muta-	The mutation rate refers to "sponta-	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	tions occur at specific sites on the DNA molecule differs for different genes.	neous" mutations, i.e., those arising with no apparent intervention on the part of the investigator. The mutation of one gene is independent of mutation in other genes.	
18.63 Mutagenic Agents	Chemical and physical agents which increase the rate of mutation by interacting with and modifying the DNA are called mutagenic agents.	Mutagenic agents are used experimentally to increase the frequency of mutation; the dose and/or time of treatment is chosen to cause approximately 90% killing of cells. No mutagenic agents are known which are specific for causing the mutation of a particular gene. General categories of mutagenic agents include the following. Substitution of DNA base analogs for normal bases results in an increased incidence of incorporation of "wrong" bases. Examples of such analogs are 5-bromouracil and 2-aminopurine. Chemicals which react with DNA bases in such a way as to change their chemical structure result in a change in hydrogen bonding properties and consequent changes in base pairing, addition, or deletion of bases. Examples of such chemicals include nitrous acid, hydroxylamine, monofunctional alkylating agent (ethyl methane sulfonate), and bifunctional alkylating agents (nitrogen mustards, mitomycin, and nitrosoguanidine). This group causes cross-linking betweeen the two DNA strands, prevents unwinding, and may result in deletion of a segment of DNA. Acridines are inserted between bases in DNA. This causes the addition or deletion of a single base, producing frame shift mutations. UV radiation exposure causes the formation of covalent bonds between two adjacent thymines on the same strand of DNA (thymine dimer), producing changes in base pairing. Ionizing radiation exposure produces multiple effects on the DNA molecule.	Comparisons of irradiated and non-irradiated cultures of *Serratia marcescens* show differences in the frequency of mutation with regard to lethal mutations, pigmentation, and colony morphology.

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18.7	Selection	Changes in the environment may give a mutant an advantage so that it will grow faster than the parent and replace it. The change in environment does not cause or induce mutations but selects for preexisting mutants.	A population tends to remain genetically stable in the absence of an environmental change selective for mutants; each mutant is in equilibrium at a frequency in the total population proportional to its mutation rate. Bacteria are more adaptable to new environments than eucaryotic cells because their haploid nature makes possible the immediate expression of mutations. In addition, bacterial growth rates are faster; and high population density increases the probability of obtaining a wider variety of mutants. Moreover, every cell can potentially give rise to a new strain. Some mutations are "selective" and confer an advantage on the organism under certain environmental conditions; others are "unselective" and confer neither an advantage nor disadvantage on the mutant cell compared to the parent. Selective mutants are easier to detect and isolate, and direct methods may be used; indirect methods for examination of very large numbers of cells is required to detect and isolate unselective mutants. Principles of natural selection as it occurs in the environment are used in developing methods of direct laboratory selection.	Two methods that can be used for the isolation of antibiotic-resistant mutants are gradient agar plates and selective media.
18.8	DNA Repair	Microorganisms have enzymatic mechanisms to repair DNA.	Photorepair or light repair mechanisms require the presence of visible light. This mechanism for repair of UV-induced damage involves breakage of the covalent bonds joining the thymine dimers; it requires a specific enzyme system. Dark repair mechanisms do not require visible light. Specific cellular enzymes excise damaged portions of a single DNA strand and other enzymes and then synthesize a segment of DNA complementary to the normal DNA strand.	Expose UV-irradiated cells to visible light and then compare frequency of mutation with cells kept in the dark after UV irradiation.
19.0	**Chemical Methods of Microbial Control**			

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19.1	Modes of Action	Microbicides are substances that kill microorganisms.	For all practical purposes, microbicides and disinfectants are synonyms for agents that are commonly used for killing of all kinds of microorganisms. However, their use is generally confined to disinfection of inanimate objects.	Laboratory experiments may be used to show that most microbicides are chemical agents that kill growing microorganisms, but not necessarily heat-resistant spores, and that many solutions of heavy salts kill a variety of microorganisms with which they come in contact.
19.11	Growth Inhibition	Microbiostatic agents are substances that inhibit the growth of microorganisms.	Chemicals commonly used as microbiostatic agents include aniline dyes, such as stains, e.g., crystal violet, which may react with cells (especially gram-positive cells) and prevent them from growing. However, cells can be quickly reactivated by washing off the dye. Other frequently used microbiostatic agents include synthetic detergents, such as quaternary ammonia compounds.	
19.12	Lethal Effects	Microorganisms may be killed by various means, including protein denaturation, cell lysis, alterations in cell membrane permeability, and interference with cellular metabolism and/or reproduction.	Solutions of heavy metals, strong acids, or halogens may precipitate protoplasmic protein (coagulation or denaturation). Coagulation of cellular protein is preceded by breakage of hydrogen or disulfide bonds in the secondary and tertiary structure of proteins (partial denaturation). Damage is irreversible. Damage to cell walls, especially those of gram-positive bacteria, may be caused by the enzyme lysozyme (found in tears, leukocytes, etc.). Disintegration of the cell wall is followed by cell lysis. Phenolic compounds, synthetic detergents, soaps, and other related substances tend to destroy the selective permeability of membranes of the cell and permit leakage of cellular constituents, especially N and P, all of which result in cell death. Interference with reproduction may be accomplished by inhibiting nucleic acid (DNA or RNA) synthesis. Such agents may inhibit nucleotide formation or interfere with polymerization of nucleotides into nucleic acid, e.g., 5-bromouracil, an antimetabolite for thymine.	Experiments may be used to show that cells subjected to certain concentrations of heavy metal solutions, phenol and phenolic solutions, high osmotic pressure-exerting solutions, synthetic detergents, and soap may no longer be able to produce colonies on suitable media.

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19.2	Factors Affecting the Action of Chemical Agents	Microbicide and microbiostatic agent effectiveness is influenced by factors such as contact time, concentration, pH, temperature, nature of microorganisms, and varying susceptibility of the agent within the target population.	As a rule, the longer the contact time with the chemical agent, the greater is the inhibition and destruction of cells. Generally, the higher the concentration of chemical agents, with some exceptions, e.g., ethyl alcohol (ETOH is most effective at a 70 to 75% concentration), the more effective is the killing or inhibition. As a rule, the lethal or toxic action of an agent is enhanced by increasing the concentration of H^+ or OH^- ions. The warmer the agent, the more effective it is because chemical reactions in general are more rapid at higher temperatures. The types, age, and numbers of microorganisms are important factors to consider. Generally, younger cells are more susceptible than older cells. Growing cells are subject to interference with metabolism, whereby nongrowing cells would not be affected. The membranes of older cells tend to be less permeable to chemical agents than the membranes of young, dividing cells. Vegetative cells of spore-forming organisms are more susceptible than dormant spore stages. In a mixed population some species are more susceptible to certain agents than others, i.e., lysozyme tends to attack gram-positive cell walls. Generally, human pathogens are more sensitive to certain agents than the normal flora of the human body. Each agent behaves differently under different circumstances.	
20.0	**Physical Methods of Microbial Control**			
20.1	Modes of Action	Physical agents may inhibit or kill microorganisms in some of the following ways: coagulation of protein, enzyme oxidation, depression of metabolism, cell hydration, and DNA injury and destruction.	Intense moist heat coagulates protein, and dry heat oxidizes protein. Damage is irreversible. Moist heat is more efficient than dry heat. Moist heat at 120 to 121°C for 15 minutes (only obtained by autoclaving) kills micro-	Experiments may be used to show that high and low temperatures, incineration, radiation, and ultrasonic waves may slow growth, inhibit, or kill microorganisms.

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		organisms, including spores, but dry heat requires a much higher temperature for a longer period (160°C for about 2 hours to sterilize laboratory glassware). Most cell enzymes are denatured at boiling temperatures. Enzymes in spores are protected from heat because of the structures of the extensive outer coverings or reduced cell water content (thick spore cortex). Temperatures below optimum for growth depress the rate of metabolism, and if the temperature is sufficiently low, growth and metabolism cease. Dessication is a process by which cell water is removed. Excessive dehydration causes cessation of metabolic activity. Loss of cell water may occur if the cells are placed in a highly osmotic solution. In such a solution cells tend to collapse (plasmolysis) due to excessive loss of cell water to the solution outside. However, freeze-drying (lyophilization) of the cell may remove water but the process does not cause injury to the cell; hence, the cell may be viable for many years. Various types of radiation are used in the control of microorganisms. UV light (260 nm) is used in killing microorganisms. Shorter waves (185 nm) are more rapidly lethal but are difficult to control and apply. Radiation such as UV, X rays, and gamma rays, tends to damage nucleic acids; such treatments either produce thymine dimers or break H bonds of nucleic acid, resulting in either the killing, injuring, or mutating of the cells. As a rule, the shorter the wavelength, the more damage to the integrity of the cells.	
20.2 Factors Affecting Modes of Action of Physical Agents	Factors affecting the modes of action of physical agents include the nature and intensity of the physical agent, exposure time, the type of microorganism, the physiological	The concentration, dosages, etc., of the physical agent have an influence on its effectiveness in the killing or inhibition of microorganisms; for example, moist heat is more	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	state of the organism, and the surrounding environment.	efficient than dry heat and gamma rays are more lethal than X rays. Contact or exposure time is inversely proportional to the intensity of the agent applied, e.g., the higher the temperature, the less time required; the shorter the wavelength, such as gamma rays, the less contact time required. If cells are surrounded by efficient heat-transferring material, they are easily inactivated or destroyed. If they are surrounded by poor heat conductors, such as soil, they tend to survive correspondingly longer.	
21.0 Antimicrobial Chemotherapeutic Agents			
21.1 Modes of Action of Chemotherapeutic Agents	Antimicrobial chemotherapeutic agents are chemical substances used in the treatment and control of various infectious diseases.	Arsenical compounds, quinine, sulfonamides, and antibiotics are used as chemotherapeutic agents, i.e., substances used in treatment of diseases by destroying or preventing the activity of parasitic organisms without major injury to the cells of the host. Historically, the chemotherapeutic agents arsphenamine and neoarsphenamine were used in control of syphilis, but they have been replaced by penicillin. Quinine and its derivatives from the bark of the chinchona tree are useful in the treatment of malaria. Antibiotics such as tetracycline and chloramphenicol are broad-spectrum antibiotics, that is, they are able to inhibit many gram-positive and gram-negative organisms. Synthetic chemotherapeutic agents are those compounds which have been found to have antimicrobial activity, for example, nitrofurans. Such agents do not occur naturally but have a broad spectrum of action. Isonicotinic acid hydrazide has been used for treatment of tuberculosis, and nalidixic acid has been used for treatment of urinary tract infections caused by gram-negative bacteria.	Laboratory experiments may be used to demonstrate the sensitivity of microorganisms to chemotherapeutic antimicrobial agents.

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21.11 Definition	In general, naturally occurring substances, as distinguished from synthetically produced compounds, are referred to as antibiotics.	Many microorganisms produce antibiotics which provide protection against other microorganisms, i.e., they excrete a specific substance that interferes with the metabolism of other microorganisms, thereby preventing their growth or killing them. Many antibiotics (naturally produced by microorganisms) are not produced synthetically on a commercial scale.	
21.12 Static and Cidal Effects	Chemotherapeutic agents can either inhibit the growth of or kill microorganisms. Those inhibiting growth are called microbiostatic agents and those killing are called microbiocidal agents.	The actions of antibiotics include the inhibition or interference with cell wall synthesis, DNA synthesis, or injury to cell membranes. Penicillin, produced by *Penicillium chrysogenum* is active primarily against gram-positive bacteria and inhibits their cell wall synthesis. Tetracycline is produced by *Streptomyces aureofaciens* and is a broad-spectrum antibiotic and interferes with protein synthesis. Novobiocin, produced by *Streptomyces griseus*, attacks primarily gram-positive bacteria and inhibits DNA polymerization. Griseofulvin produced by *S. griseus* attacks pathogenic fungi and interferes with fungal cell wall and nucleic acid synthesis. Other microbiostatic agents include sulfonamide drugs which act as structural analogs of paraaminobenzoic acid. Many different cellular enzymes (enzymes in the glycolytic system, citric acid cycle, and cytochrome system) are potential targets for an inhibitor. For example, cyanamide inhibits glycolysis; trivalent arsenic compounds may block the tricarboxylic acid cycle; and many halogens (Cl, B, I) and other oxidizing agents may alter or inactivate the enzymes.	
22.0 Introduction to Immunity (Resistance)			
22.1 The Nature of Immunity	Immunity refers to the ability of an individual organism to resist and/or overcome		

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		a disease agent to which most or many of its species are susceptible.		
22.11	The Immune Response	The immune response is a specific adaptive response to foreign substances and materials which include infectious disease agents, certain chemicals, and cells. There are two types of immunity, cellular and humoral, which may aid in eliminating foreign material or rendering it harmless.		
22.12	Lymphocytes	Immunity depends upon specifically reactive white blood cells called lymphocytes. Most lymphocytes are found in the spleen, lymph nodes, and Peyer's patches.		
22.13	B- and T-Type Lymphocytes	Lymphocytes, which are responsible for humoral immunity (the production of antibodies), are referred to as bone marrow or B cells. Those cells responsible for cellular immunity and influenced by the thymus gland are called T cells (Medical Microbiology, Subtopic 14.14).		A diagram showing the origins of T and B cells and their functional interactions may be used. Scanning micrographs of these cells also may be helpful.
22.2	Antigens (Immunogens)	Antigens are substances which, when introduced into a host, will provoke a detectable immune response.		
22.21	General Properties	Antigens are perceived as foreign to a host's immune system. The host is normally tolerant (nonreactive) to its own tissues and does not consider them as foreign.		
22.22	Chemical Composition	Some antigens are proteins, whereas others may be polysaccharides. Nucleic acids and certain lipids also are antigenic in some cases (Medical Microbiology, Subtopic 14.22).		
22.23	Antigenic Determinants	Antigenic determinants are those parts of the antigen molecule that are actually involved in binding with antibody (Medical Microbiology, Subtopic 14.23).		

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22.24	Haptens	A hapten is generally a small molecule which is incapable of initiating an immune response. Such molecules can become antigenic through linkage with large carrier molecules (Medical Microbiology, Subtopic 16.24).		
22.25	Examples of Antigenic Materials	Bacteria (or their parts) or virus particles may serve as antigens. Most exotoxins are proteins. Their toxoids (altered toxins), which have little or no toxic properties but retain antigenicity and antigenic determinants, upon injection bring about the formation of antitoxins. These molecules serve to protect against the effects of toxins. Microbial antigens, such as whole cells (killed or attenuated) or viruses, when injected into animals including humans, induce a response resulting in the formation of specific immunoglobulins.		
22.3	Immunoglobulins (Antibodies)	Antibodies or immunoglobulins represent a specific group of heterogeneous proteins which are formed in response to antigenic stimulation and react specifically with the antigen that provoked their formation. Immunoglobulins are found in body fluids such as blood, lymph, and tissue fluids.		
22.31	Classes of Immunoglobulins	Five major classes of human immunoglobulins are recognized, each with unique chemical properties and functions. The five classes are termed immunoglobulin G (IgG), IgA, IgM, IgD, and IgE (Medical Microbiology, Subtopics 14.34–14.367). Most classes of immunoglobulins have been further divided into subclasses.		
22.32	Mechanism of Action	Antibodies may interfere with or inhibit the activities of foreign cells and substances through various mechanisms, including increased opsonization, which facilitates the phagocytosis of microorganisms and other materials; cellular destruction (cytolysis) of		

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		certain microorganisms and other cells in the presence of complement (a system of serum proteins which is activated by certain antigen and antibody reactions); neutralization of toxins; and prevention of the attachment of microorganisms such as viruses to susceptible host cells.		
22.4	Host-Parasite Relationships	Organisms live in various relationships to each other in nature. In the human body, host-parasite relationships may exist in several body sites. One of the undesirable relationships is parasitism. This is the association in which one organism lives either on or in another form of life (the host) at the expense of the host.		
22.41	Host Resistance	The ability of a host to prevent or to overcome the effects of a disease-causing microorganism (pathogen) is related to an individual's immunity. Such an ability to resist pathogens may be attributed to two factors: natural resistance (factors inherent in the body) or resistance acquired during life (acquired immunity).	Natural resistance of a host depends on a large number of factors, including the host's genetic constitution, general health, nutrition, and environment and social conditions. Acquired resistance is usually due to the individual's experiences of infections and immunizations (Introductory Microbiology, Topic 23.0, and Medical Microbiology, Subtopic 14.5).	
22.43	Host Defense Mechanisms	Invading pathogens must be capable of overcoming various anatomical and physiological barriers of the host's body before they can establish an infectious process.	Body defense mechanisms include physical barriers (intact skin), cellular factors, or biochemical factors (antigen-antibody reactions) (Medical Microbiology, Subtopic 13.5). Physical resistance mechanisms include intact skin, mucous membranes, and all cases of flushing or clearing of the invading microorganisms from the anatomical site. Phagocytosis by macrophages incorporates both physical and biochemical mechanisms. Biochemical factors include reactions involving various enzymes, body fluids, interferon and antibodies with invading microorganisms, or those reactions between microbial toxins and antitoxins. The normal flora of the human body,	

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		which is found on the skin and mucous membranes and other locations without causing disease, also contributes to protecting the host against pathogens (Medical Microbiology, Subtopic 13.3).	
22.5 Microbial Virulence Factors	The disease-producing capacity or virulence of organisms is determined by genetic properties which may be fully expressed under certain environmental conditions. This virulence may be due to various factors, including an organism's ability to produce toxic substances (toxigenicity), invasiveness, and ability to survive within a host.	Pathogens which fully express virulence may be able to grow and multiply, increase in local lesions, or spread throughout the host, be able to tolerate phagocytic action of leukocytes, be able to prevent production of antibodies, and be able to produce substances injurious to host tissues (Medical Microbiology, Subtopics 13.65–13.67).	
22.51 Microbial Toxins	Microbial toxins are poisonous substances produced by various microorganisms. Examples of such toxins include exotoxins, enterotoxins, endotoxins, and mycotoxins (Medical Microbiology, Subtopics 13.63–13.634).	Toxins interfere with normal cellular activities.	
22.52 Invasiveness	Invasiveness is the ability of an organism to spread among the tissues of a host. This virulence factor involves not only counteracting normal host defenses but being able to spread from the original site of penetration.		
23.0 States Of Immunity And Diagnostic Immunology			
23.1 Cell-Mediated Immunity	Cell-mediated immunity is regulated by T-type lymphocytes and is dependent on the presence of the thymus gland at birth. Immune responses mediated by T-type lymphocytes are important in combating fungal parasites, chronic bacterial infections, and certain viral infections and are responsible for many accelerated rejections of allografts (grafted cells or tissues from genetically different individuals of the same species) (Medical Microbiology, Subtopic 14.4).		

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23.11	T-Cell Sensitization	A T cell which comes into contact with an antigen for which it is specific is said to be committed or sensitized. Sensitized T cells multiply after exposure to such antigens and give rise to clones of cells, which produce a variety of biological effects.	Sensitized T cells provide protection to the host through cellular mechanisms such as promoting phagocytosis (by producing lymphokines such as chemotactic factor and macrophage-activating factor) preventing viral multiplication via interferon, and contracting and destroying tumor cells and other foreign cells, including virus-infected cells. In cell-mediated immunity, the antigen may combine with particular lymphocytes (activated), and such cells enlarge, divide, and provide mediators (lymphokines) which induce local inflammation and participate in the elimination of the foreign material. A classical example of this response is the immunity to tubercle bacilli (Medical Microbiology, Subtopic 14.43).	
23.2	Humoral Immunity	Humoral immunity is mediated by antibodies. B-type lymphocytes are responsible for antibody production.	Individuals who have recovered from measles or mumps or who have been immunized with diphtheria, pertussis, and tetanus (DPT) antigens are protected from these diseases because they have produced specific antibodies against each of the causative agents and/or toxoids. Certain disease states are diagnosed in the laboratory by demonstrating the presence of specific antibodies in a patient's serum, e.g., syphilis and infectious mononucleosis.	
23.21	Primary Response	The immune response that occurs after initial exposure to a given antigen is called the primary response. With humoral immunity, IgM is usually the first antibody produced and is detectable in about 1 week.		
23.22	Secondary Response	Upon reexposure to the same antigen used in an initial exposure, the body produces a greater antibody response in a shorter period of time, known as the secondary or anamnestic response. IgG, which is produced in this response, is detectable for		

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	long periods and is associated with a long-lasting protection (Medical Microbiology, Subtopics 14.512–14.514).		
23.3 General States of Immunity	Native and acquired immunities are recognized as general states of immunity.		
23.31 Native Immunity	Native immunity refers to various factors involved with species resistance, racial resistance, and individual resistance.	Resistance to infection varies with the species of animal or plant; e.g., infectious diseases of cold-blooded animals, with a few exceptions, rarely occur in warm-blooded animals. Racial resistance refers to genetic factors which influence disease resistance or susceptibility and is an inherited trait, e.g., Native Americans are less resistant to tuberculosis than Caucasians. The principle of species resistance is extensively used in agriculture—the breeding of resistant plants is essential in crop production.	
23.32 Acquired Immunity	Immunity resulting from an exposure to antigen is said to be acquired. Such immunity to a specific antigen may be actively or passively acquired.		
23.321 Active vs. Passive Forms	Active immunity may develop from an infection or artificially by an immunization procedure such as vaccination. The immune response here is produced by the host. In passively acquired immunity, the immune response is not produced by the host. Preformed antibodies may be acquired naturally from other sources (Medical Microbiology, Subtopics 14.5–14.52).		
23.33 Types of Acquired Immunity	Acquired immunity may be of four basic types: naturally acquired active, naturally acquired passive, artificially acquired active, and artificially acquired passive.	Examples of the four basic types of immunity include the following. Naturally acquired active: natural exposure to an infection. Naturally acquired passive: placental transfer of IgG. IgA in the colostrum of mother's milk. Artificially acquired active: injection or other artificial application of attenuated microorganisms, or toxoids. Ar-	

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		tificially acquired passive: injection of antibody-containing serum from another individual or animal.	
23.4 Immune Disorders and Hypersensitivity	Immune disorders result from immune deficiencies such as lack of functional B-type lymphocytes, or T-type lymphocytes, or both.	Certain individuals are unable to combat certain infectious agents because they lack either humoral immunity and/or cell-mediated immunity. Examples of primary immunodeficiency include the following: Lack of functional B lymphocytes results in poor humoral immunity but normal cellular immunity; Lack of functional T lymphocytes results in poor cellular immunity and some decrease in humoral immunity (where T-lymphocyte cooperation is involved in antigen recognition); Lack of functional T lymphocytes and B lymphocytes results in little or no humoral immunity or cellular immunity. As a rule, the immune system may decrease in function with advanced age.	
23.41 States of Hypersensitivity	Hypersensitivity or allergy may result from adverse effects of humoral immunity (immediate hypersensitivity) or cell-mediated immunity (delayed hypersensitivity). Clinically, the states of hypersensitivity are organized into type I (anaphylaxis), type II (cytotoxic reaction), type III (immune complex reaction), and type IV (delayed hypersensitivity) (Medical Microbiology, Subtopics 14.61–16.64).	Hypersensitivities can be experimentally shown in laboratory animals, e.g., anaphlaxis and graft rejection. In cases of immediate hypersensitivity or allergy, the production of antibodies against antigens causes more damage to the host than good. Reactions of this type can be observed within minutes to hours after exposure to allergens. There are three mechanisms of immediate hypersensitivity: (i) IgE mediated (anaphylactic type of type I), (ii) antibody-dependent cytotoxicity (type II), (iii) immune complex mediated (type III). In type I, antigen-antibody reactions bring about the massive release of histamine and related substances which trigger inflammation as well as cause the constriction of smooth muscles in veins, arteries, and bronchioles; hence, immediate injections of anti-	

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		histamines may be necessary to alleviate the symptoms of the hypersensitive reaction. Type I hypersensitivity reactions may be induced by the inhalation of ragweed pollen or the ingestion of certain foods. These substances react with IgE molecules attached to mast cells in the tissues or to basophils in the bloodstream, and thereby bring about the symptoms of allergy. "Allergy shots" are administered to allergic individuals to stimulate production of specific antibody (IgG) in their serum. IgG antibody neutralizes the allergen and is given little or no opportunity for contact with the mast cell-attached IgE. Diagnostic skin tests such as those used as a presumptive test for tuberculosis are based on delayed hypersensitivity. Delayed hypersensitivity (type IV) does not involve circulating antibodies (the same mechanism as cellular immunity). Responses are usually seen within hours to days after exposure to allergens.	
23.5 Diagnostic Testing	Various diagnostic procedures are used widely to demonstrate the presence of both antigens and antibodies in body fluids and/or tissues. The results of such tests are of medical (clinical) importance to the identification of infectious agents, some chemicals, and various cellular antigens or their corresponding antibodies. In all diagnostic immunological tests, the identity of one component, antigen or antibody, is known.		
23.51 Identification of Antigens	An unknown antigen can be identified or typed by means of known antibody.		
23.52 Identification of Antibody	The presence or absence of antibody and the titer (level) of antibody can be determined by employing a known antigen-antibody reaction. (Medical Microbiology, Subtopics 20.2–20.23).		

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23.53	Immunological Tests	A wide range of tests and variations of these tests exist to detect specific antigens or antibodies in specimens. These include agglutination, precipitation, immunofluorescent-antibody techniques, radioimmunoassay (RIA), enzyme-linked immunoabsorbent assay (ELISA), and skin tests. (Medical Microbiology, Subtopics 20.31–20.4).		
24.0	**Epidemiology and Infectious Diseases**			
24.1	Principles of Epidemiology	Epidemiology is a branch of science that deals with the causes of disease and with the factors that influence the distribution of disease-producing agents within populations.	Factors that influence the distribution of disease-producing agents (pathogens) within a population include changes in the immunity status, age profile, and vector characteristics of the host population. Epidemiological studies associated with infectious diseases include portal of entry and exit, survival rate outside of host, carrier state, microbial population invasiveness, toxin production, plasmid transfer, and host population. The interaction of host and parasite populations, geographical distribution, prevalence of disease, seasonal distribution, etc., also are significant. The suspected causative agent of an infectious disease must be isolated and identified by laboratory tests or incriminated strongly by specific symptoms of infected individuals. The presence of appropriate vehicles such as fomites and/or vectors in the environment will enhance the distribution of pathogens. Three interconnected factors, host, agent, environment determine the severity of epidemics caused by microorganisms. A successful pathogen may produce disease, but usually does not kill the host.	
24.2	Mode of Transmission	For a pathogen to cause a disease, it must travel from habitats in a given reservoir, be transported to a susceptible host, and colo-	Infected animals, humans, and contaminated objects may serve as reservoirs of disease-causing agents.	

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	nize or exhibit its virulence within a new host.	Infectious agents may be transmitted to susceptible hosts by direct contact or by vectors such as arthropods. Cysts and spores of microbial pathogens enable infectious agents to survive adverse conditions that would be destructive to vegetative cells during dispersal from reservoir to susceptible host. Bacterial endospores are more resistant to destruction by antimicrobial agents than are vegetative cells. Protozoan cysts, such as those of *Giardia lamblia*, are not destroyed by chlorine at concentrations used in municipal water purification facilities. The environmental conditions associated with a susceptible host influence the manner in which the disease agent interacts with host tissues.	
24.21 Direct and Indirect Contact	Processes that involve the transmission of microorganisms include two broad groups of mechanisms, namely, direct contact and indirect contact.	Direct transmission involves events such as biting, licking, kissing, and those which involve an exchange of mucus secretions between an infected person or animal and a susceptible host. Infectious agents are transmitted to susceptible individuals by mechanical and biological means. Mechanical means include contact with contaminated inanimate objects (fomites), nuclei of mucus and saliva, water, air, soil, food, dairy products, sewage, etc. (indirect transmission). A nosocomial infection (hospital-acquired infection) is a good example of contact transmission of a pathogenic agent. Here as well in other situations, the agent may be acquired from air, food, or contaminated apparatus or directly from infected individuals. Biological means of disease transmission involve living vectors that transmit pathogens directly from one susceptible host to another. Certain infectious diseases primarily as-	

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			sociated with lower animals may be transmitted to humans by a variety of vectors (mosquitoes, ticks, fleas, etc.). Such diseases are examples of zoonoses. A portal of entry to the body will often determine the nature and severity of a disease Syphilis may be acquired through sexual contact with an asymptomatic individual having a symptomatic or asymtomatic form of the disease (direct contact transmission). Diphtheria may be acquired by inhaling droplets (nuclei of mucous secretion droplets containing virulent infectious agents) expelled into the air, through sneezing or coughing, etc. (indirect contact transmission). Rabies is most frequently spread to humans and other mammals by the bite or the saliva of an infected animal.	
24.3	Representative (Infectious) Microbial Agents	Pathogenic agents have varying abilities to injure body tissues and/or alter body functions by a variety of mechanisms.	Virulence factors include: adherence to host cells; tissue affinity (certain microorganisms have a particular affinity for certain cells and tissues which they may injure or destroy); toxic factors; the production of exo- or endotoxins or both (exotoxins as a rule are more detrimental to the host than endotoxins); enzymic and other factors contributing to virulence (e.g., production of hyaluronidase [spreading factor], lecithinase [alpha-toxin, which destroys red blood cells], collagenase [destroys collagen]; coagulase [clotting factor]; fibrinolysin [streptokinase, dissolves human fibrin]; leukocidin [kills leukocytes in vitro]; hemolysin [liberates hemoglobin from red blood cells]); and capsular material (encapsulated strains of certain bacteria are pathogenic, whereas noncapsulated strains are nonenpathogenic, but there are exceptions).	
24.31	Infectious States and Intoxications	Infectious diseases are caused by pathogens from the following microbial groups:	Many pathogens have a rather specific host range.	

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	bacteria, fungi, protozoans, viruses, and the multicellular worms or helminths. Some microbes such as algae and certain bacteria cause noninfectious diseases states such as poisonings (intoxications).	Certain bacteria, fungi, and viruses may be capable of infecting more than one kind of animal host. As a rule, most plant pathogens are nonpathogenic to animals and vice versa. Bacteria, algae, and fungi share a similar molecular basis of disease, i.e., produce toxins and enzymes causing injury to the cells or tissues. Gonorrhoea caused by *Neisseria gonorrhoeae* and syphilis (*Treponema pallidum*) are examples of bacterial diseases. Histoplasmosis (caused by *Histoplasma capsulatum*), and coccidiomycosis (*Coccidioides immitis*) are examples of systemic mycoses or deep-seated fungal diseases. Protozoans may invade the body cells and tissues, multiply within them, and cause destruction of cells and tissues, which may result in a variety of diseases. Malaria (caused by *Plasmodium vivax*) and amoebic dysentery (*Entamoeba histolytica* infection) are examples of protozoan diseases. Hepatitis (caused by type A and type B viruses) and German measles (caused by rubella virus) are examples of viral diseases. Trichinosis or pork roundworm (caused by *Trichinella spiralis*) and infection with tapeworms (*Taenia saginata*, beef tapeworm; *T. solium*, pork tapeworm) are examples of helminth infections.	
24.4 Control Measures	Infectious diseases are controlled by processes that involve one or a combination of the following: chemotherapy, prophylactic immunization (passive measures), and environmental sanitation.	Prophylactic measures may involve immunization or the administration of chemotherapeutic agents in certain extreme cases. Many viral diseases are controlled by extensive immunization programs (poliomyelitis, influenza, measles, mumps). Smallpox has been eradicated by means of a massive immunization campaign. DPT vaccines are administered to children to protect them from diphtheria, whooping cough, and tetanus.	

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		Those infectious diseases requiring a specific vector may be controlled by the eradication of the vector (yellow fever, malaria, plague, etc.). Antiseptics are used to reduce the number of pathogens on host tissues, and disinfectants are used to remove and destroy pathogens on inanimate materials. Some diseases are controlled by chemotherapy and the detection and treatment of carriers since immunization against such diseases is ineffective, e.g., syphilis and gonorrhoea. Certain viral diseases are controlled by the elimination of carriers and prophylactic immunization (rabies), whereas some animal diseases are controlled by the elimination of diseased individuals as well as carriers (foot and mouth disease). Typhoid fever, for example, is largely controlled by good environmental sanitation, whereas poliomyelitis is controlled by immunization and environmental sanitation. Diphtheria may be controlled by prophylactic immunization and improved environmental sanitation. Postinfection measures may involve different forms of immunization materials, the use of chemotherapeutic agents, and improvements in environmental sanitation.	
25.0 Environmental Microbiology			
25.1 Sanitation Microbiology	Microorganisms are an integral part of air, water, and solid waste pollution. Microorganisms are ubiquitous. Polluted air, water, and land almost always contain numerous types of organisms. Some of these organisms are pathogenic to plants and to animals, whereas others are saprophytes involved in decomposition of pollutants. **Taber, W. A.** 1976. Wastewater microbiology. Annu. Rev. Microbiol. **30**:263–277.	Most airborne bacteria are transient, i.e., no bacteria use air as a habitat. Likewise, solid waste may contain a heterogeneous group of organisms, whereas water or wastewater may contain indigenous as well as transient populations of organisms. Increased organic loading of lakes, streams, and estuaries has taxed the natural system to recycle the waste material, resulting in pollution. Water pollution can be detected by indicator species (i.e., coliforms)	Laboratory examination of a variety of samples may be used to show the presence of microorganisms.

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		and other biological and chemical tests, i.e., biochemical oxygen demand (BOD) and chemical oxygen demand (COD). Open dumping of solid waste in a community is an open invitation to the spread of disease by flies and rats. Solid waste, which may be constituted of fibers, metals, and animal and human wastes, may contain a number of pathogens. Improperly disposed liquid or solid wastes may produce not only a health hazard but undesirable odors from decomposition as well. Decomposition of buried waste (sanitary landfill) involves anaerobic microbial activity. Properly buried material is slowly decomposed to various compounds or elements and recycled into the environment without causing undue harm.	
25.11 Decomposition Activities	Microorganisms serve a vital function in the elimination of some environmental pollutants from air, water, and land. **Crites, R. W., and C. G. Pound.** 1976. Land treatment of municipal wastewater. Environ. Sci. Technol. **10**:548–551.	Microorganisms play an indispensible role in the decomposition of organic materials found in source water (water for drinking purposes). Pathogenic bacteria are eliminated by chlorination or ozonation after physical-chemical treatment.	
25.2 Aquatic Microbiology	Microorganisms are present at all depths in the aquatic environment. No natural aquatic environment is devoid of organisms. Many such microbes are introduced by air or soil, from surrounding environment, or by human activity.	Microorganisms are principal agents in biological wastewater treatment (trickling filters, activated sludge processes). The organisms stabilize noxious material as well as material which may fertilize receiving bodies of water. Pathogenic organisms are destroyed before water is discharged into receiving bodies by various disinfection processes, e.g., chlorination, ozonation, etc. Microorganisms may be distributed in all parts of a lake. The littoral zone (along the shore with good light penetration to the bottom) harbors more bacteria per milliliter than other parts of the lake because the nutrient concentration is higher. In the limnetic zone (open area: depth and turbidity determine the amount of light	

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			penetration), the largest population of microorganisms is found in the top centimeters, and it supports both autotrophic and heterotrophic organisms. In the profunal zone (deeper regions of open water) the population of microorganisms is smallest. The benthic zone (bottom area: little or no light, a zone of decomposition) harbors large numbers of microflora and -fauna. Many are anaerobic, active decomposers of the sedimented organic material. Aquatic microorganisms may be divided into three groups: (i) Planktonic (floating and drifting organisms, mostly algae [phytoplankton] and protozoans [zooplankton]). (ii) Nektonic (swimming organisms). (iii) Benthic (bottom swellers). Indigenous microorganisms may be photosynthetic, chemoautotrophic, or chemoorganotrophic. Hot springs may contain thermophilic organisms able to tolerate a temperature as high as 90°C.	
25.21	Food Chains	Micoorganisms form the basis of activities such as food chains and decomposition cycles within aquatic environments.	Photosynthetic microorganisms (blue-green bacteria and others) may function as primary producers in the food chain, whereas other microorganisms serve as decomposers within the aquatic environment. The components of a pond food chain include: primary producer (plankton algae) → primary consumer species (minnows or small fry of larger fish) → secondary consumer species (bluegill or catfish) → tertiary consumer species (bass or pike) → dead plants and animals attacked by decomposers (bacteria and aquatic fungi).	
25.3	Soil Microbiology	The soil serves as a major habitat for many autotrophic and heterotrophic micro-	The soil contains water and minerals, in addition to organic material from the re-	Laboratory techniques may be used to determine microbial numbers within soil.

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		organisms. Soils are formed as a result of geological and biological processes.	mains of dead plants, animals, insects, and humus. It is a dynamic habitat, a constantly changing microbial population. Distribution and function of soil microorganisms: (i) Bacteria and other microorganisms degrade complex organic compounds in the rhizosphere. (ii) Fungi serve as active decomposers, major "crumb" formers. (iii) Algae fix carbon dioxide and contribute to soil fertility. (iv) Protozoans are the prime predators. The soil harbors many different nutritional types of microorganisms. Some are photoautotrophs, some are chemolithotrophs, and the great majority are chemoorganotrophic saprophytes. Some exist symbiotically, whereas others exist in a free-living state.	Topsoil may harbor a billion organisms per gram.
25.31	Microbial Activities	Soil microorganisms play an essential role in the recycling of nutrients.	Both autotrophs and heterotrophs in soil serve as biogeochemical agents for the mineralization of organic C, N, S, P, and other compounds. Metabolic activities microorganisms include the following. Transformations of nitrogen: organic nitrogen → ammonia → nitrites → nitrates. Transformations of sulfur: sulfates → hydrogen sulfide → elemental sulfur → sulfates → organic sulfur → hydrogen sulfide. Transformations of carbon: carbon dioxide → organic carbon → carbon dioxide.	
26.0	**Industrial Microbiology**			
26.1	Food Microbiology	Microorganisms are used in the manufacture of various foods, vitamins, drugs, and chemicals. **Ryther, J. H., and J. C. Goldman.** 1975. Microbes as food in maricultures. Annu. Rev. Microbiol. **29**:429–443.	A special strain of yeast is used in preparation of dough to produce CO_2, which causes the dough to rise. Sauerkraut, pickles, sausage, and other fermented foods are produced by special kinds of bacteria which are able to tolerate high osmotic environments and to produce	Various foods may be used to demonstrate microbial processes. Microorganisms are used in bakeries and other food industries for the preparation of sausage, sauerkraut, and pickles.

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			large amounts of lactic acid and other flavor-enhancing products. Lactic acid produced by the organisms serves as a food preservative. Single-cell microorganisms are used as sources of protein supplements in food for domestic animals. The raw materials used for microbial cultivation are generally waste products such as spent paper mill water (sulfite liquor) or whey, blanching water, etc. These microorganisms decompose and stabilize wastes and serve as a source of protein.	
26.11	Food Texture and Flavor	Microorganisms are used in the food industry to enhance the taste, flavor, texture, and nutritive value of foods.	Microorganisms impart specific or unique taste, flavor, and texture to food, but they also increase its digestability and nutritive value. A gram of yeast cells may provide not only protein but almost all essential vitamins and growth factors for animals, including humans.	Aroma and taste of freshly baked bread are attributed to the activities of yeast cells. The flavor of fermented foods is due to the products of bacterial metabolism.
26.12	Spoilage Organisms	Microorganisms also are capable of food spoilage.	In food microbiology, time, money, and effort are spent in protecting food from microbial spoilage and elimination of pathogens. Some of the methods used in protecting food from spoilage include: aseptic handling of food, pasteurization, boiling and steam sterilization; low temperature (refrigeration, freezing), dehydration, increasing osmotic pressure (concentrated sugar or salt), presence of preservative chemicals (organic acids produced from fermentation and various products formed during smoking), and radiation (ultraviolet and ionizing). Because foods are handled by many people, they are subject to contamination with a variety of pathogens and frequently cause outbreaks of food poisoning and food associated infections.	Perishable foods such as fresh fruit, vegetables, fish, poultry, and meat eventually decompose when they are left at room temperature. Such foods can be shown to contain a wide variety and large numbers of bacteria.
26.2	Dairy Microbiology	The preparation of butter, cheeses, and fermented milk products such as buttermilk	Bacteria which produce large amounts of lactic acid (strains of *Lactobacillus* and	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		and yogurt depend on the metabolic activities of particular microorganisms.	*Streptococcus*) are used as starter cultures for many fermented milk products. Cheeses, butter, sour cream, buttermilk, acidophilus milk, yogurt, kefir, and kumiss are produced by fermentation of milk. Special bacteria and fungi are used in the manufacture of milk and dairy products. Ripening of some cheeses depends on specific kinds of bacteria, whereas other cheeses require molds, e.g., bleu cheese, roquefort, etc. Fermented milk and milk products have a longer keeping quality than raw milk, since they contain a variety of organic acids produced by bacteria. Pasteurization is used to protect the public from milk-borne diseases (similar to foodborne), such as salmonellosis, tuberculosis, brucellosis, and Q-fever.	
26.3	Industrial Application of Microorganisms	Microorganisms are utilized in the production of alcohols, amino acids, antibiotics, enzymes, hormones, vitamins, vaccines, and other commercially important products. **Kleyn, J., and J. Hough.** 1971. The microbiology of brewing. Annu. Rev. Microbial. **25**:583–608.	Microorganisms used industrially include molds, yeasts, and bacteria. Pathogenic microorganisms and their products are used to prepare vaccines. Most processes using raw materials and microorganisms are carried out by using the continuous culture method (chemostat) rather than batch method (Microbial Physiology, Subtopic 3.4). Microorganisms employed in the manufacture of products are constantly improved by various genetic manipulations. Some bacteria are used in steroid conversions. Many industrial processes involving microorganisms utilize waste materials (whey, corn steep liquor) to manufacture useful and essential products.	
27.0	**The Future of Microbiology**			
27.1	Genetic Engineering	The manipulation of genetic material to obtain desired results is referred to as genetic engineering. Genetic engineering can	The ability to produce certain desired products (antibiotics, organic acids, interferon, insulin, alcohols, etc.), the ability to	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	be a powerful tool to construct organisms that may have agricultural, industrial, or medical importance.	decompose certain materials, or the ability to produce toxic substances is controlled by genes which may be manipulated.	
27.11 The Use of Microorganisms	Artificially created recombinant DNA molecules can be replicated in bacteria such as *Escherichia coli*. Genes of higher organisms coding for insulin, interferon, and other useful products can be inserted into plasmids which, when introduced into microorganisms, replicate and produce functional products.		
27.12 Scientific Concerns	Some scientists are concerned that recombinant DNA technology may result in unexpected biological hazards. **Grobstein, C.** 1977. The recombinant DNA debate. Sci. Am. July. **237**:22-33.		
27.2 Factors Affecting the Incidence of Infectious Diseases	Infectious diseases have become less prominent causes of death and disability in regions of improved sanitation and adequate sources of antibiotics. However, the misuse of antibiotics has resulted in an increase in the incidence of antibiotic-resistant microorganisms.	Conjugal transfer of functional plasmid(s), such as toxic factors (either exo- or endotoxins or both) and enzymic factors (R-factor), has created considerable problems in chemotherapy and control of gonorrhea and other assorted diseases. Acquisition of genes for pili (structures that facilitate adhesion to target tissue) or the ability to produce capsules may enhance the virulence of such organisms.	
27.3 Microbial Applications to Future World Problems	Microorganisms have played and will continue to play important roles in resolving world problems including environmental pollution and food shortages. **Kihlberg, R.** 1972. The microbe as a source of food. Annu. Rev. Microbiol. **26**: 427–466.	Microorganisms may be used as protein supplements, to control pests in crop production, to reduce or eliminate famines, to control water and soil pollution, and to recover valuable products from wastes for recycling to conserve raw material. Single-cell microbes such as yeast cells can be harvested from wastes from home and industry. Waste celluloids may be solubilized by an enzyme produced by organisms which will convert it to sugar. Sugar can then be used to grow yeast, which can be consumed by humans directly or indirectly	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		as a source of protein and vitamins or added to animal feed as a supplement. Microorganisms, e.g., *Bacillus thuringinesis* and some viruses, rather than biologically recalcitrant chemicals such as DDT, have been used to control crop-damaging insects. This keeps the environment free of insecticides. Solid wastes may be composted to fertilizer or soil conditioner or fermented to recover fuel (CH_4, CH_3OH, C_2H_5OH) for home and industrial use.	
27.4 Evolution of Microorganisms	Genetic changes occur among members of a species through modification of DNA (mutation). Mutants, either spontaneous or induced, are subject to the same selective pressure as nonmutants. Mutation results in increased physiological versatility when mutants thrive better or can survive better a particular environment.	The ecosystem (biotic and abiotic components) provides internal as well as external selective pressures. Most ecosystems are variable. Variation may be natural, induced, or brought about by internal or external forces. These include frequent changes in temperature, pH, available water and nutrients, amount of radiation received, antibiotics produced, or toxic substances introduced by humans or by flora and fauna within and external to the given environment. Such variables may be selective forces. Examples would include the following: bacteria endowed with resistance (R) plasmids are more apt to survive in the environment containing antibiotics; lowering the pH tends to favor acid-tolerant organisms.	
27.41 Causes of Genotypic Changes	Genotypic changes in cells also may result from processes such as conjugation, transformation, and transduction.	Alteration in genomes may be mediated by lysogenic viruses. Many bacteria are capable of conjugally transferring functional plasmids such as R factor to members of their genus and to members of other genera.	
27.5 Microbiology Specialties	Opportunities at various levels exist for individuals educated and well-trained in the basic sciences and the application of microbiological principles and techniques.	Microbiologists are employed in the following specialty categories: Agricultural microbiology: cause-effect control of plant pathogens, improvement of	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		soil fertility, nitrogen fixation, ecto- and endosymbiosis, etc. Aquatic and marine microbiology: microbial ecology-relationship of organisms to other organisms or flora and fauna and to abiotic components (beneficial-detrimental effect), and pollution control. Food microbiology: preservation of food (prevent spoilage); storage; improvement in nutritional value, aroma, flavor, taste, and texture; quality control; food sanitation. Dairy microbiology: manufacture of cheeses and other fermented milk products, quality control, epidemiology of certain microbial diseases of cows. Sanitary or public health microbiology: the study of epidemiology of food- and water-borne infections or diseases; maintain bacteriological safety of potable water and swimming pools; and examination of effluent from wastewater treatment. Medical microbiology: epidemiology, pathogenesis, immunology, chemotherapy, and diagnostic microbiology. Industrial microbiology: search for and development of antimicrobial agents, production of biochemicals, and vaccines, and recombinant DNA applications.	
27.51 Factors Affecting Employment	Salaries and employment, as in other professions, are dependent on the level and extent of education, training, and experience.		

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1.0	**Survey of the Biological World**			
1.1	Taxonomy	Biological classification or taxonomy is the systematic arrangement of organisms into groups or categories called taxa according to a definite scheme.		
1.2	Classification	Organisms may be classified on the basis of several characteristics, including morphology, staining reactions, physiological properties, chemical structure, and antigenicity.		
1.3	Nomenclature	Nomenclature entails the systematic and scientific naming of organisms.		
1.31	Genus and Species	The scientific name of an organism consists of genus and species (binomial system of nomenclature). A genus represents a group of closely related species, but a species represents one kind of organism.		
1.32	Names of Organisms	The first name of an organism is the genus, the second is the species. The first letter of the genus is capitalized, whereas the first letter of the species is not. The scientific name of an organism is printed in italics or is underlined.	Both names of an organism frequently are descriptive, i.e., *Micrococcus* (a small grain) *albus* (white), a spherically shaped bacterium which produces white colonies.	
1.4	The Five-Kingdom Approach to Classification (Other Approaches Exist)	Based on their characteristics, organisms may be grouped into one of the following five kingdoms: Animal, Plant, Fungi, Protista, and Monera. The five kingdoms are differentiated primarily on the basis of morphology, motility, and mode of obtaining energy.	Since viruses are not cellular, they are not included in this classification scheme.	
1.41	Animals	The animal kingdom includes organisms that are multicellular, eucaryotic, and nonphotosynthetic; lack cell walls; have tissue differentiation; and ingest their nutrients.	Most animals are motile, lack cell walls, possess a mouth and a digestive cavity, and utilize organic materials for food and energy.	
1.42	Plants	The plant kingdom includes organisms that are multicellular, eucaryotic, photosynthetic, and have tissue differentiation.	Most higher plants are non-motile, possess a cell wall, and are differentiated into tissues, roots, stems, and leaves. All plants	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			absorb their nutrients from their surroundings.	
1.43	Fungi	Fungi are non-photosynthetic unicellular or multicellular organisms which obtain their nutrients by absorption.	Most fungi are heterotrophic (use organic compounds as sources of carbon) and are aerobic saprophytes.	
1.44	Protista	The protista consist of eucaryotic, predominantly unicellular organisms that include algae and protozoa.		
1.45	Monera	The monera consist of unicellular, procaryotic organisms (all bacteria).		
1.5	Viruses	Viruses are submicroscopic obligate intracellular parasites. Since they do not have cellular organization, they are not considered true cells.		
2.0	**Microorganisms**			
2.1	The Nature of Microorganisms	Microorganisms are microscopic or submicroscopic forms of life and can be fixed (immobilized) on glass slides and stained with various aniline dyes to demonstrate specific distinguishing structures and properties. Compound light microscopes are used to observe most microorganisms in living and stained preparations.	The smallest organism that can be seen with the naked eye is approximately 0.1 mm. The smallest organism that can be seen with a light microscope is approximately 0.2 μm. Bacteria are usually in the range of 1 μm. (One micrometer [μm] equals 10^{-6} meters.)	
2.12	Microbial Organisms	Viruses, bacteria, most fungi, protozoa, and certain algae represent the five general forms of microorganisms. Since algae are seldom of medical importance, they are not considered in medical microbiology.	Microorganisms have a wider range of physiological and biochemical potentialities than all other organisms combined. There are few fields of human endeavor where microorganisms do not play an important role. Only a small percentage of microorganisms are capable of growing or surviving on or in the human body.	Microscope and projection slides showing characteristic examples of the five forms of microorganisms may be studied.
2.13	Reproduction	All microorganisms except viruses are capable of reproduction either asexually and/or sexually. Viruses and certain bacteria (rickettsia and chlamydia) must grow inside living cells to reproduce.	Asexual reproduction involves replication of chromosomal deoxyribonucleic acid (DNA) before division into two cells. Sexual reproduction of microorganisms requires transfer of genetic material from one cell to another.	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
2.2	Concept of the Cell	The cell is the basic structural unit of life and is composed of: cell wall (if present), cell membrane, cytoplasm, and nuclear material (chromosomes).		Diagrams showing structural units of microorganisms may be studied.
2.3	Single-Cell Organisms	Most microorganisms are unicellular organisms which are capable of independent existence.	A single-celled organism is capable of carrying out all the processes associated with life within one cell; these microorganisms are quite complex physiologically despite their small size.	Projection slides of bacteria and protozoans prepared for light and/or electron microscopy may be studied to show basic structures.
2.4	Procaryotic and Eucaryotic Microorganisms	There are two types of cellular organization: procaryotic and eucaryotic (Introductory Microbiology, Table 1).		
2.41	Procaryotes	Among other characteristics, procaryotic microorganisms lack a nuclear membrane and membrane-bound organelles. Bacteria are procaryotic, whereas fungi and protozoa are eucaryotic.		Diagrams of bacteria and protozoans may be studied to demonstrate cell structure.
2.42	Eucaryotes	Among other characteristics, eucaryotic organisms possess a defined membrane-bound nucleus and other organelles which may be visible with a light microscope in stained or unstained preparations.	Eucaryotic microorganisms are structurally more complex than procaryotic organisms.	
2.5	Viruses	Viruses are not cells since they do not possess a cell membrane or nuclear material capable of self replication. Viruses are, therefore, neither eucaryotic nor procaryotic (Medical Microbiology, Topic 8).		
3.0	**Microscopy and Stains**			
3.1	Microscope	A microscope is an instrument consisting of a combination of lenses to make small objects such as microorganisms look larger in order that they may be seen and studied. The ability to distinguish two distinct points as separate entities is the resolving power of a microscope.	The ability to see microorganisms with the aid of a microscope paved the way for the establishment of the germ theory of disease.	A chart comparing relative sizes of objects after magnification may be studied.
3.2	Types of Microscopes	There are many types of microscopes. The		

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		choice of microscope depends on the nature of the specimen and the information required.		
3.21	Bright-Field Microscope	The most commonly used microscope to observe microorganisms is a bright-field microscope in which stained organisms appear dark against a bright background. Bright-field microscopes generally magnify up to 1,000 times. The resolving power of a bright-field microscope is 0.2 μm.	The parts of a bright-field microscope include the ocular (top lens system), objective (lower lens system), mechanical stage, condenser, and light source. The magnification of a light microscope may be determined by multiplying the magnification of the ocular by that of the objective. Viruses and certain bacteria cannot be seen with the aid of a bright-field microscope because they are smaller than the resolving power of the microscope.	A bright-field microscope may be studied and used by all students. Some objects such as stained yeast cells may be observed at all the available magnifications.
3.22	Dark-Field Microscope	In dark-field microscopy, incoming light is directed so that the organisms appear light in a dark background. Dark-field microscopy is often used to observe viable organisms and organisms slightly below the resolving power of a bright-field microscope. No stains are required.	*Treponema pallidum*, the causative agent of syphilis, is usually examined under dark-field illumination.	A diagram showing the principles of dark-field microscopy and microscope slides of bacteria observed with a dark-field microscope may be studied.
3.23	Phase-Contrast Microscope	Phase differences occur between light altered by an object and the unaltered background light. Differences in the density or chemical composition of the organisms are contrasted by phase variation against the background. No stains are required.	The principles of phase microscopy dictate that when light passes through microorganisms, it emerges in different patterns depending on the different properties of the material through which it passes. Internal structures of living microorganisms are studied in this manner.	Microscope slides showing internal structures of eucaryotic microorganisms may be observed by phase-contrast microscopy.
3.24	Fluorescence Microscope	The second emission of visible light by an object such as a bacterium stained with a fluorescent dye and illuminated with light of short wavelength such as ultraviolet is fluorescence. Fluorescence microscopes require special optics and light sources.	The identification of certain pathogenic microorganisms or the presence of specific antibodies is often determined with the aid of fluorescence microscopy and specific antibody coupled to a fluorescent dye. For example, rabies virus may be identified in a rabid animal's brain tissue by use of the fluorescent microscope.	Projection slides showing fluorescing bacteria identified by specific fluorescent antibody may be shown.
3.25	Electron Microscope	Electrons produce shorter wavelengths than light. Instead of light, an electron mi-	Electron microscopes aid in the study of viruses and have greatly added to our un-	A diagram showing the components of an electron microscope along with electron mi-

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	croscope uses beams of electrons projected through a vacuum and focused by electromagnets for viewing on a fluorescent screen. The electron microscope can resolve particles 0.001 μm apart.	derstanding of the morphology of microorganisms.	crographs of microorganisms may be studied.
3.3 Stains Used with Bright-Field Microscopes	Most microorganisms are transparent and must be stained with dyes before they can be seen under the microscope.	Many bacteriological dyes are coal tar derivatives known as aniline dyes.	The value of staining may be demonstrated by examining stained and unstained bacterial smears.
3.31 Differential Stains	Certain staining procedures such as the Gram stain and acid-fast stain separate organisms into categories, i.e., gram-positive or gram-negative, acid-fast or non-acid-fast. This is often the first step in identification of bacteria.	Differentiation occurs because of differences in the chemical contents of the various types of cells.	Gram and acid-fast staining should be performed if possible. Microscope slides or projection slides of stained organisms may also be studied.
3.311 Gram Stain	The Gram stain is the most prevalently used stain for bacteria. Gram-positive bacteria retain crystal violet upon decolorization and appear purple. Gram-negative bacteria do not retain crystal violet upon decolorization. They are then stained red by a safranin counterstain.	Several mechanisms for the Gram reaction have been proposed; however, it is still poorly understood.	
3.312 Acid-Fast Stain	The acid-fast stain is primarily used to detect mycobacteria which retain a red dye complex due to their high lipid content even after decolorization with acid alcohol. Non-acid-fast organisms do not retain the complex but may be visualized after counterstaining.	The agent of tuberculosis, *Mycobacterium tuberculosis* is a classic example of an acid-fast microorganism.	
3.313 Special Stains	The staining of spores, flagella, and capsules is performed to promote visibility of the respective structures and to aid in categorization and identification.		Microscope slides of bacteria stained with special stains are available commercially and should be studied.
3.4 Clinical Application	Specimens from patients are often Gram or acid-fast stained and observed under the brightfield microscope to provide the clinician with information regarding the presence of microorganisms, their relative concentrations, and their staining reactions.		

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		This information is helpful regarding treatment and management of the patients.		
4.0	**Metabolism and Growth**			
4.1	Cellular Composition	All living things, including microorganisms, are composed of protoplasm. The common chemical elements carbon (C), oxygen (O), hydrogen (H), and nitrogen (N), compose about 99% of protoplasm. These chemical elements are arranged into complex organic molecules such as proteins, carbohydrates, lipids, and nucleic acids, which are necessary for the microorganism to live and reproduce. A basic knowledge of cellular composition and metabolism is necessary to understand the concepts of medical microbiology		
4.2	Metabolism	Metabolism is the sum of all the chemical reactions occurring within an organism. They involve a breakdown of large organic molecules into simpler ones (catabolism) with the liberation of energy necessary for the organism's activities and the build-up of simple molecules to complex ones (anabolism) necessary for the organism to carry out these activities.	The breakdown of molecules does not produce energy but transforms it from one form to another.	
4.21	Organic Molecules	All living material contains organic molecules. All organic molecules contain carbon (Microbial Physiology, Topic 1).		
4.211	Proteins	Proteins are very large complex organic compounds composed of numerous small molecules called amino acids. Proteins are important as enzymes and as structural elements of cells.	There are about 20 different amino acids. Thousands of amino acid molecules may be combined to make proteins.	
4.212	Carbohydrates (Sugars)	Carbohydrates are compounds that contain only carbon, hydrogen, and oxygen. They may be small molecules (monosaccharides) or large complex molecules (polysaccharides) used as structural elements of the cell or as a stored energy source.		

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
4.213	Lipids (Fats and Related Compounds)	Lipids are composed of carbon, hydrogen, and oxygen but also may contain other elements such as phosphorus and nitrogen. They are insoluble in water and are important in intermediary metabolism and in structural elements such as the cell membrane.		
4.214	Nucleic Acids	Nucleic acids are compounds that play a major role in the transmission of hereditary traits, in the control of cell functions, and in the synthesis of proteins.		
4.3	Heterotrophs	Heterotrophic organisms obtain their carbon requirements from organic molecules. Heterotrophs use organic molecules both as a source of carbon and as a source of energy. Pathogenic microorganisms are heterotrophs as are most animals. Many nonpathogenic microorganisms obtain their carbon from carbon dioxide (CO_2). These organisms are autotrophic as are plants.		
4.4	Enzymes	The transformation of energy necessary in catabolism and anabolism is carried out in part by enzymes. Enzymes are organic catalysts, i.e., substances which in minute amounts promote chemical changes without being used up in the reactions. A substrate is the substance, e.g., an organic molecule, which is permanently altered in an enzyme-catalyzed reaction (Microbial Physiology, Topic 4).	Enzymes and substrates are highly specific for each other. Enzymes are named according to the type of reaction catalyzed (e.g., hydrolase) or the substrate acted upon (e.g., protease). Certain bacteria produce enzymes which relate directly or indirectly to pathogenesis. Lecithinase, the alpha toxin of *Clostridium perfringens* causes lysis of the host cell membranes.	
4.41	Exoenzymes	Many microorganisms secrete enzymes into the external environment (exoenzymes) that split large molecules into smaller ones which can enter the cell.	Exoenzymes hydrolyze starch to glucose and proteins to amino acids. Determination and identification of exoenzymes often aid in the identification of microorganisms. Many bacterial virulence factors, including toxins, are exoenzymes.	
4.5	Small Molecules	Small molecules for anabolism are obtained from catabolism and from outside the cell. Small molecules outside the cell may be		

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	passively (diffusion) or actively transported inside the cell by enzyme-catalyzed reactions.		
4.6 Metabolic Pathways	A particular series of stepwise chemical reactions resulting in a given end product or products is called a metabolic pathway. Glucose is a simple 6 carbon sugar used as an energy source by many microorganisms, since it is widely distributed in nature and is a part of many larger molecules. The breakdown of glucose into smaller molecules by various metabolic pathways is a major energy source and produces smaller molecules as by-products (Microbial Physiology, Subtopics 5.2 and 5.3).		
4.61 ATP	Adenosine triphosphate (ATP) is a molecule which is important in energy transfer. ATP is formed from adenosine diphosphate (ADP) as a result of catabolic reactions. Energy is released by breaking the high energy bond of ATP (ATP → ADP + Pi). Such energy is available for biosynthesis and other energy-requiring reactions.		
4.7 Fermentation and Respiration	Fermentation and respiration are the two basic catabolic schemes found in microorganisms. Fermentation involves energy production without the use of molecular oxygen, whereas respiration requires it. Fermenters are divided into organisms which cannot tolerate the presence of molecular oxygen (anaerobes) and those which can (facultative anaerobes) (Microbial Physiology, Subtopics 5.2 and 5.21).	Organisms which use molecular oxygen in respiration are more energy efficient and produce more molecules of ATP per molecule of glucose catabolized. Therefore, they usually have shorter generation times. The determination of an organism's catabolic scheme and the end products of the scheme are used in the identification of microorganisms.	
4.8 Quantitation of Microorganisms	In the clinical laboratory, it is often important to know the amount (number of organisms per milliliter) of a given organism or the percentage of a given organism in a mixed population. Microorganisms may be quantitated by several means; however, in the clinical bacteriology laboratory, count-	Abnormal amounts or increased percentages of an organism may indicate a disease state, e.g., greater than 10^5 bacteria per ml of urine generally indicates disease.	

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	ing, which determines the number of viable bacteria, is generally used.		
4.81 Generation Time	Although growth refers to an increase in the volume of protoplasm, with microorganisms it generally refers to an increase in cell numbers (reproduction). The average time needed for one microorganism to divide into two is its generation time, e.g., *Escherichia coli* = 17 minutes, enabling large populations of cells to be produced in a short time (Microbial Physiology, Topic 3 and Microbial Genetics, Subtopic 1.2).	The ability of pathogenic microorganisms to rapidly reproduce enables them to colonize a host and produce disease in relatively short periods of time. In nature, growth of microorganisms is slowed due to the accumulation of their own waste materials, depletion of nutrients, and their inability to adjust to changes in environment. The growth of pathogenic microorganisms in a host is also slowed by the host's defense mechanisms.	
4.9 Media	To study microorganisms such as bacteria in the laboratory, nutrients and environment must be provided which are conducive to growth and reproduction. "Medium" is a general term used to denote nutrient materials used for the culture of microorganisms. The proper temperature, pH, oxygen tension, and other environmental factors must also be provided to insure growth.	The ideal medium and environment should duplicate those conditions in nature in which a given microorganism thrives. Media must be sterile or free from microorganisms whose development might influence or prevent the normal growth and metabolism of the inoculated type. Sterilization of media is usually by means of autoclaving or in certain cases by filtration.	
4.91 Uses of Media	Media may be used for isolation, identification, and maintenance of microorganisms. Media may be liquid, solid, or semi-solid.		
4.92 Agar	Agar, used most frequently as a solidifying agent at 1.5 to 2.0% for media, is a polysaccharide obtained from seaweed. It produces a substance the consistency of gelatin. It is not metabolized by most bacteria, liquefies at 100°C, and solidifies at 45°C. Nutrient material necessary for metabolism is incorporated in the agar.	Agar plates, i.e., agar medium in a petri dish, provide ideal media for isolation of bacterial colonies and for study of colonial characteristics, such as color, shape, size, and consistency.	Agar plates and slants may be prepared as a laboratory exercise. Plates and slants may then be inoculated with a microorganism such as *E. coli.*
4.93 Complex and Synthetic Media	Complex media are those for which the chemical composition is not defined, e.g., blood and milk. Media in which all the components are known, in kind and amount, are synthetic.	Growth factors are small compounds that certain bacteria cannot synthesize and therefore must be added to the media.	

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4.94 Differential Media	Differential media are used to more easily detect colonies of bacteria which have distinctive properties such as hemolysis of blood, gas production, or acid production.	A differential medium is one which will cause the colonies of a particular organism to have a distinctive appearance, e.g., *E. coli* has a characteristic irridescent sheen on EMB (eosin-methylene blue) agar.	
4.95 Selective Media	An ideal selective medium is one that inhibits the growth of all microorganisms in a specimen and supports the growth of a desired species or other defined group. Selective media are formulated by: inclusion of inhibitory substances; establishing extremes, e.g., pH and salt concentration; or the presence or absence of growth factors.	In clinical laboratories, media used are often selective and differential.	
4.96 Enrichment Media	An enrichment medium is one to which special nutrients have been added to enhance the growth of certain microorganisms, e.g., blood agar.	Many bacteria isolated from clinical specimens require blood or blood products and increased CO_2 tension. These conditions are more reflective of the natural environment.	
5.0 Genetics			
5.1 Genetics	Genetics is the study of the mechanisms by which the information of cells is stored, expressed, and modified and of how this information is transmitted to future generations of organisms and to other cells in the population.		
5.2 Genetically Important Molecules	The major molecules important in the study of genetics are deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein (Microbial Genetics, Topic 2).		
5.3 DNA	DNA is a double-stranded molecule in the form of a double helix, each strand of which is a polymer of nucleotides. DNA is the reservoir of genetic information in a cell (Microbial Genetics, Subtopic 2.1).	A nucleotide consists of deoxyribose sugar, a phosphate group, and one of four bases: adenine, guanine, thymine, or cytosine.	
5.31 DNA of Procaryotes	DNA of procaryotes is a circular molecule located in the cytoplasm and is not surrounded by a membrane. This molecule is called a chromosome and contains segments	A fully extended bacterial chromosome is about 1 mm long.	Electron micrographs may be used to illustrate the circular nature of DNA.

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		called genes. Genes determine the characteristics of cells and act as the control units of all cellular activity.		
5.32	DNA of Eucaryotes	DNA of eucaryotes is distributed into chromosomes. A eucaryotic chromosome is composed of one very large DNA molecule, histone proteins, and other proteins. The chromosomes are located in the membrane-bound nucleus.	The number of chromosomes found in the nucleus varies among eucaryotic organisms. Human body cells contain 46 chromosomes, an onion has 16, but procaryotic cells have only 1.	
5.33	Gene	A gene is the part of the DNA molecule which carries the genetic information for the synthesis of one protein.	Genes code for the enzymes which regulate all the chemical reactions of the cell.	
5.34	Genome	The entire complement of genes in an organism comprises its genome.		
5.35	DNA Function	DNA has two major functions: to duplicate itself and to code for synthesis of protein.	DNA is replicated so that each daughter cell receives an exact copy of the genetic material.	
5.36	DNA Replication (Duplication)	The two DNA strands separate. Enzymes catalyze the synthesis of new strands of DNA complementary to each of the original two strands. The two new DNA molecules formed are identical to the original.	Each strand of the original molecule serves as a template resulting in two DNA molecules having one strand from the original DNA and one strand of newly synthesized DNA.	
5.361	Mitosis	Mitosis is the separation of previously replicated chromosomes of eucaryotic cells.	Mitosis occurs in all animal and plant cells.	
5.362	Meiosis	Eucaryotic cells, which have a sexual cycle, carry out a reduction-division process (meiosis) which results in one diploid cell becoming four haploid cells. Diploid cells contain a pair ($2n$) of each chromosome, whereas haploid cells contain half (n) the number of chromosomes as diploid cells.		
5.363	Procaryotic Cell Replication	Procaryotic cells do not undergo mitosis or meiosis. The circular DNA is replicated, and each cell produced by asexual cell division has one DNA molecule as did the parent cell. The result is two equal daughter		

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	cells which are replicas of the parent (binary fission) (Microbial Genetics, Subtopic 2.2).		
5.4 RNA	RNA is a single-stranded polymer of nucleotides. It differs from DNA in that the sugar ribose replaces deoxyribose and the base uracil replaces thymine.		
5.41 RNA Synthesis	RNA molecules are enzymatically synthesized as complements to one strand of a particular region of DNA. RNA molecules are temporary and are constantly synthesized.		
5.42 Types of RNA	There are three types of RNA: messenger RNA (mRNA); transfer RNA (tRNA); and ribosomal RNA (rRNA). They function at various stages of protein synthesis.		
5.5 Protein Synthesis	Genetic information flows from DNA to mRNA to protein. Each codon, a sequence of three nucleotides, specifies a particular amino acid. Special codons specify sites of initiation or termination of a protein (Microbial Genetics, Subtopic 2.3).	Each amino acid may be coded for by several different codons.	
5.51 Transcription	The mRNA molecule is synthesized as a complement to a gene on one strand of DNA. The sequence of the bases in mRNA determines the amino acid sequence of a protein.	A specific mRNA is a transitory molecule involved in the synthesis of one specific protein or portion of a protein. mRNA may be copied several times during the synthesis of a protein.	
5.52 Translation	The mRNA molecule is decoded on the ribosome by the tRNA molecules carrying amino acids. Each tRNA attaches to a specific amino acid and also recognizes one specific codon of the mRNA. The ribosome moves along the mRNA, resulting in the sequential polymerization of amino acids to synthesize a protein.	Several ribosomes may move along one mRNA at the same time. This results in a polysome, which appears as a cluster of ribosomes on an electron micrograph.	
5.6 Mutation	A mutation involves an inheritable change in the base sequence of DNA. A mutation may change any characteristic of a cell or		

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	virus. There are different types of mutations (Microbial Genetics, Topic 4).		
5.7 Phenotypic Expression	Most mutations result in the change of a protein. Mutations may or may not cause an observable change in phenotype, depending upon the extent or site of change.	The following are examples of phenotypic changes: structural—loss of ability to produce a capsule; nutritional—loss of ability to synthesize an enzyme resulting in a requirement for a particular nutrient or growth factor; and drug or virus resistance—ability to grow in the presence of a drug or virus which kills or inhibits growth of the parent.	
5.71 Mutation Rate	The frequency with which mutations occur at specific sites on the DNA molecule is referred to as the mutation rate. Mutation rate expresses the probability that a cell will undergo mutation at a particular gene.		
5.72 Spontaneous Mutation	A spontaneous mutation arises without apparent intervention. An average value for frequency of spontaneous mutation per gene is one mutation in 10^6 cells.		
5.73 Mutagenic Agents	Agents which interact with and modify DNA and thereby increase the rate of mutation are mutagenic agents. Examples of mutagenic agents include irradiation, DNA base analogs, and certain chemicals which react with DNA in such a way as to change its chemical structure.		
5.74 Selection	Changes in the environment may give a mutant an advantage so that it will grow faster than the parent and replace it. The change in environment does not cause or induce mutation but selects for preexisting mutants.	An example of natural selection is the increased incidence of penicillin-resistant *Staphylococcus* strains after initiation of penicillin therapy in the 1940s. This mutation was not induced by penicillin, but penicillin acted as a selective agent for preexisting mutants.	Antibiotic-containing media can be used to select antibiotic-resistant mutants in a population. The replica-plating technique can be used to demonstrate that the mutants were preexistent and not induced by the antibiotic.
5.8 Transfer of DNA Between Procaryotic Cells	DNA is transferred from donor to recipient cells by three different mechanisms. Once inside the recipient cell, the donor DNA may recombine with the recipient chromosome (Microbial Genetics, Subtopic 5.3).		

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5.81	DNA-Mediated Transformation	In DNA-mediated transformation, DNA fragments released from cells that lyse are taken up by recipient cells (Microbial Genetics, Subtopic 5.31).		
5.82	Conjugation	In conjugation, the DNA may be transferred by means of sex pili. The donor DNA fragment is transferred during the time the donor and recipient cells are in contact (Microbial Genetics, Subtopic 5.32).		
5.83	Transduction	In transduction, the donor DNA is transferred from the donor bacterium to a recipient bacterium by a bacteriophage (virus which infects a bacterial cell) (Microbial Genetics, Subtopic 5.33).		
5.9	Plasmid	A plasmid is extrachromosomal circular DNA which may exist independently in the cytoplasm or become integrated into the procaryotic cell chromosome (Microbial Genetics, Subtopic 3.12).	Plasmids may be very important in transferring drug resistance among bacteria.	
5.91	Plasmid Transfer	An entire plasmid may be transferred by any of the three mechanisms for DNA transfer (Microbial Genetics, Subtopic 3.34).	Many plasmids have wide host ranges and may be transferred between different genera.	
5.92	Lysogeny	Some bacterial viruses integrate their nucleic acid into the DNA of the cells they invade. The viral nucleic acid is replicated along with the bacterial DNA (Microbial Genetics, Subtopic 5.12).	*Corynebacterium diphtheriae* strains only cause diptheria when lysogenized by a specific bacteriophage. Scarlet fever is caused by a lysogenic strain of *Streptococcus pyogenes*.	
6.0	**General Characteristics of Bacteria**			
6.1	Shape	Most bacterial species exhibit one of three general forms; cylindrical (rods or bacilli); spherical (coccus); or spiral (spirillum).		Microscopic slides containing stained preparations of bacteria having these shapes may be observed under the microscope.
6.11	Arrangement	Although bacteria are unicellular, the individual cells may not separate completely after cell division and therefore exhibit a variety of cellular arrangements. Depending on the plane of division, cocci may be ar-	Cellular arrangements often aid in the classification and identification of bacteria. When bacteria are found in a patient's specimen, the Gram reaction, cellular shape, and cellular arrangement are often reported to	Typical arrangements may be observed under the microscope using stained preparations on microscope slides.

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		ranged in pairs (diplococci), chains (streptococci), fours (tetrads), cuboidal packets (sarcina), or clusters (staphylococci). Rods may appear singly, form chains, line up in parallel (palisades), or form unusual letter patterns (X, Y, or Z).	the clinician before the organism is identified, because they provide valuable information regarding disease.	
6.12	Colony	Bacteria grown on solid culture media (agar) multiply repeatedly to form a visible structure called a colony. A colony is theoretically a clone of bacteria resulting from the multiplication of a single organism, that organism is referred to as a colony-forming unit.	Colonial morphology is often used as an aid in identification and classification. Colonies differ in many characteristics, including size, shape, color, and texture.	Various species of bacteria streaked for isolation on agar media may be studied for colonial morphology.
6.2	Surface-Associated Structures			
6.21	Capsule	Some species of bacteria are surrounded by a viscous layer termed a capsule. Capsules are important in medical microbiology since they aid in protecting bacteria from engulfment (phagocytosis) by host cells and thus enhance the bacteria's ability to produce disease.	Capsular antigens are also used for serological identification (typing) of bacteria in the clinical laboratory (Medical Microbiology, Subtopic 15.212).	Projection slides of organisms such as *Streptococcus pneumonia* and *Cryptococcus neoformans*, specially stained to show their capsules, may be shown.
6.22	Flagellum	Some bacteria produce long appendages called flagella which serve in locomotion. Flagella are composed of protein subunits arranged in a helix. The various arrangements in which flagella occur are useful in classifying certain bacteria (Microbial Physiology, Subtopic 6.22).	The flagellar or H antigens are often used for serological typing of certain bacteria.	Microscopic slides of specially prepared bacteria such as *Proteus vulgarus* with stained flagella may be observed under the microscope.
6.23	Pilus	Certain bacteria, especially freshly isolated, gram-negative rods, possess submicroscopic appendages known as pili. They are thinner, shorter, and more numerous than flagella and are not involved in motility. One kind of pilus (sex pilus) is involved in the transfer of genetic material from one bacterium to another during conjugation.	Piliated bacteria tend to attach to other objects such as inert surfaces, living cell surfaces, and other bacteria.	Electron micrographs of organisms with pili may be studied.
6.24	Cell Wall	Bacteria possess a rigid cell wall which gives the bacteria their shape (rod, coccus,	Mycoplasmatales are the only family of bacteria which do not possess a cell wall.	

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		spiral). Gram-positive bacteria have a thick cell wall composed almost entirely of a complex polymer called a peptidoglycan. The cell wall of gram-negative bacteria is composed of peptidoglycan, lipopolysaccharide, and protein. Lysis of gram-negative bacteria releases the cell wall components, which are now called endotoxin, and produce disease symptoms.	Wall-defective microbial forms (WDMF) are bacteria which have lost some or all of their cell wall. Certain antibiotics and enzymes produce WDMFs. Protoplasts, spheroplasts, and L forms are specific types of WDMFs.	
6.26	Cytoplasmic Membrane	The cytoplasmic membrane is a thin semipermeable membrane immediately beneath the cell wall. It regulates the passage of material between the cell and its environment.	The cytoplasmic membrane is similar to the eucaryotic cytoplasmic membrane and is also called the plasma or cell membrane.	
6.261	Mesosomes	Mesosomes are internal extensions of the cytoplasmic membrane which are associated with cross-cell wall formation at the time of cell division. They are unique to bacteria.		
6.27	Cytoplasm	The cytoplasm includes all the contents within the cell membrane. It is granular in appearance and rich in RNA and ribosomes.		
6.28	Nuclear Region (Nucleoid)	The nuclear region is composed primarily of DNA and carries the complete genetic information of the bacteria. It is equivalent to the nucleus of eucaryotic cells but consists only of a single circular DNA molecule (chromosome).	Many bacteria contain circular extrachromosomal strands of DNA called plasmids. A plasmid may exist independently in the cytoplasm or may become integrated into the chromosome.	
6.29	Endospores	Certain bacteria produce highly resistant and refractile, thickwalled, oval bodies termed endospores, which enable them to survive harsh environments. Endospores are highly resistant to physical and chemical treatment. When the environment becomes favorable, each endospore yields a single vegetative bacterium.	Endospores of certain bacteria such as the clostridia are commonly found in the soil. Endospores of the *Clostridium* spp. causing tetanus and gas gangrene may germinate in dirty wounds leading to disease production. The resulting vegetative bacteria produce exotoxins responsible for the disease state.	
6.3	Classification	Bacteria are divided into 19 categories based on readily determinable criteria. This	The description of the 19 categories is given in *Bergey's Manual of Determinative*	

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	division avoids confusion in assigning organisms to discrete classes.	*Bacteriology* (8th ed., Williams and Wilkins Co., Baltimore, 1974), which is the standard reference on classification of bacteria.	
7.0 General Characteristics of Fungi			
7.1 Fungi	The fungi are non-photosynthetic eucaryotic microorganisms having a cell wall and usually growing either as branching tubular filaments (hyphae) which form a mat (mycelium) or as unicellular yeasts. The cell size is larger than that of bacteria.	Hyphae grow by elongation at their tips and by producing side branches. The hyphae may be aerial and bear sexual or asexual spores, or may be vegetative and extend into the medium to provide the mycelium with nutrients and water. Yeasts reproduce by budding.	Moldy bread or fruit, or a portion of a yeast cake may be examined in the laboratory.
7.11 Importance or Relevance	Fungi may be saprophytic (living on dead organic material) or parasitic (living in or on another organism).	Saprophytic fungi are important in breaking down organic materials in nature. Plant pathogens such as rusts and smuts are fungi. Other fungi may be parasites of animals including humans.	
7.12 Molds and Yeasts	Molds are fungi which produce mycelia. A few fungi are unicellular, oval or spherical, do not form true mycelia, and are called yeasts.	Molds may produce mycelia which are compact and tough or loose and fluffy. Yeast colonies usually appear as thick, moist, pasty-looking colonies.	Tube cultures of mycelial (*Aspergillus*) fungal growth can be compared with that of a yeast (*Saccharomyces*).
7.2 Classification	Fungi are classified as Phycomycetes, Ascomycetes, or Basidiomycetes on the basis of their type of sexual reproduction. A fourth group, the Deuteromycetes (Fungi Imperfecti) either have no sexual stage or the sexual stage has not yet been found.	In the Phycomycetes, two compatible cells fuse to form a zygospore. In the Ascomycetes, sexually produced spores (ascospores) are contained within the ascus. Sexually produced spores (basidiospores) are borne in the basidium in the Basidiomycetes.	
7.21 Morphological Types of Molds			
7.22 Molds with Nonseptate Hyphae (Phycomycetes)	Multinucleate molds without cross-walls that produce asexual spores in a sporangium are the Phycomycetes.	The nonseptate (coenocytic) molds characteristically have wider hyphae and grow faster than other molds.	*Mucor* or *Rhizopus* may easily be cultured as examples of coenocytic molds.
7.23 Molds with Septate Hyphae	Most molds have hyphae divided by septae, or cross-walls, which extend in from the periphery of the hyphae. These include Ascomycetes, Basidiomycetes, and Deuteromycetes.	The hole in the middle of the cross-wall allows nutrients, nuclei, and cell organelles to pass through from one cell to another.	*Aspergillus* and *Penicillium* may be cultured as examples of molds with septate hyphae.

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7.3	Reproduction	Molds reproduce naturally by spore formation, but in addition most parts of these fungi are capable of growth after being fragmented.	While fungi are classified on the basis of their sexual spores, the sexual stages are difficult to induce, so the description of fungi is based principally upon their asexual structures.	Slide cultures may be prepared by using bits of mycelium as the inoculum. The common mold *Penicillium* may be used.
7.31	Spores	Asexual spores which vary greatly in color, size, and shape are produced on specialized hyphal stalks. Morphology and mode of origin of the asexual spores constitute the main basis for classifying fungi that lack sexuality. Sexual spores (haploid cells from the parents) fuse to give a diploid cell. After meiosis haploid spores are produced again.	Asexual spores (5 to 20 μm) are formed in large numbers, light-weight, easily disbursed into the environment, and usually provide the typical color of the mycelium.	
7.311	Conidiospores (Conidia)	Conidiospores (one type of asexual spore) which occur singly or in groups are born externally at the tips or sides in a hypha called a conidiophore. Some species have two types of conidia differing in size and structure and are designated microconidia and macroconidia.	The dermatophytes *Trichophyton*, *Microsporum*, and *Epidermophyton* are differentiated by the presence or absence and type of micro- and/or macroconidia (Medical Microbiology, Subtopic 16.3).	Conidia of *Aspergillus* and *Penicillium* may be studied in the laboratory.
7.312	Thallospores	Thallospores are formed by segmentation of the mycelium in actively reproducing cells. These include arthrospores (oidia) which are fragmented segments of septate hyphae and blastospores which are produced by yeast cells.	*Coccidioides immitis* produces arthrospores which may remain dormant in the arid countryside until the next rain activates them. (Medical Microbiology, Subtopic 16.51).	*Geotrichum* produces arthrospores which may be studied in the laboratory.
7.313	Chlamydospores	Chlamydospores are thick-walled, enlarged, resting spores formed within hyphae or terminal cells. Chlamydospores are more resistant to heat and to drying than other parts of the mycelium or other fungal spores.	Chlamydospores are formed when growing conditions become less favorable, whether cultured in the laboratory or in the human body.	Chlamydospores can be readily seen in most old cultures, for example with *Candida albicans*.
7.314	Sporangiospores	Sporangiospores are asexual spores borne internally inside a sac and are characteristic of the Phycomycetes.	The sac readily ruptures and releases spores into the culture tube, or into the air.	*Mucor* or *Rhizopus* spores may be easily studied in slide culture.
7.4	Yeast Morphology and Reproduction	Yeasts are oval, spherical, or cylindrical unicellular organisms with rigid cell walls; they normally reproduce by budding.	Certain yeasts may also reproduce by fission to yield sexual haploid yeast cells.	

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7.41	Pseudohyphae	Some yeasts and their progeny under special cultural conditions or in the body adhere to each other and form a chain called pseudohyphae.	The ability to form pseudohyphae helps to differentiate species of yeasts.	
7.42	Capsules	Certain yeasts are covered with a capsule e.g., *Cryptococcus neoformans.*	The presence of the capsule makes phagocytosis of pathogenic encapsulated yeasts difficult and they are therefore more virulent.	
7.5	Classification of Yeasts	Although yeasts exhibit uniformity of morphology, they do not form a natural taxonomic group and are found in three classes of the fungi. The taxonomy of most yeasts is based upon biochemical tests.	Yeasts may be members of the Ascomycetes, Basidiomycetes, or Deuteromycetes.	Projection slides of the biochemical reactions of various yeasts may be shown.
7.6	Dimorphism	Some fungi (dimorphic) can grow in either the form of molds or yeasts depending upon the environment.	Many fungal pathogens are dimorphic and may be differentiated from nonpathogens by their ability to form yeasts at 37°C and molds at room temperature.	
7.7	Cultural Characteristics of the Fungi			
7.71	Growth	Fungi are heterotrophic and will grow on ordinary laboratory media.	Fungi can grow on almost any medium and normally grow more slowly than bacteria. Sabouraud agar is the most common fungal medium.	Plates of Sabouraud agar will readily support the growth of common laboratory fungi.
7.72	O_2 Requirements	Fungi are usually strict aerobes, but some yeasts are facultative anaerobes.	*Saccharomyces* and other yeasts grown aerobically produce CO_2 from glucose and may be used in baking bread. The same yeasts grown anaerobically produce alcohol and CO_2 from glucose and are used in beer and wine making.	
7.73	Temperature	Fungi grow over a wide temperature range from 0 to 62°C. The normal range is 20 to 30°C.	Fungi grow at refrigerator temperatures and may be found growing in improperly disinfected laboratory incubators.	
7.74	Moisture	Fungi require at least a slightly moist environment for growth, but the spores may survive a more arid environment.		

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7.75 pH	Fungi have a pH range from 2.2 to 9.6 but most fungi grow best at about pH 6 to 7.	Media used to isolate fungi from a mixed culture are usually pH 5.6 or lower so that bacteria will be inhibited while the molds flourish.	Media prepared with different pH ratings can be used to compare the growth effects on a given fungus.
7.76 Osmophilic Nature	Many fungi are osmophilic, i.e., able to tolerate high salt or sugar concentrations.	Fungi grow easily on jelly or jam and will grow on hams preserved with salt.	Growth of fungi and bacteria may be compared on 50 and 100% syrup.
7.8 Fungal By-Products			
7.81 Antibiotics	Certain fungi produce antibiotics, e.g., penicillins and cephalosporins.	Useful antibiotics are produced by some *Penicillium* and some *Cephalosporium* species.	
7.82 Mycotoxins	Some fungi produce poisonous substances, termed mycotoxins, which are highly toxic.	Aflatoxin is a mycotoxin produced by *Aspergillus flavus* grown on corn or peanuts which were improperly harvested or stored. Mycotoxin has been shown to be carcinogenic in laboratory animals.	
8.0 General Characteristics of Viruses			
8.1 Physical Characteristics	Viruses are the smallest known infectious agents and contain either RNA or DNA (never both) as their genetic material (genome). They are noncellular chemical particles and obligate intracellular parasites.	The nucleic acid (DNA or RNA) of a given virus may exist as either a single-stranded or double-stranded molecule.	Diagrammatic sketches of different types of viruses showing nucleic acid, capsid symmetry, and envelope configuration may be studied. Electron micrographs depicting actual viruses are also available.
8.11 Capsid	The capsid is the protein shell which encloses the nucleic acid of the virus.	The capsid protects the nucleic acid and facilitates attachment of the virus to its host cell.	
8.111 Capsomer	The capsomer is the basic morphological repetitive polypeptide unit that collectively forms the capsid.		
8.12 Nucleocapsid	The capsid with the enclosed nucleic acid is called the nucleocapsid.		
8.13 Envelope	Many viruses have an external envelope surrounding the capsid. It is lipid or lipoprotein and is acquired in part from host cell membrane. Viruses that do not have envelopes are termed naked.	Some enveloped viruses have projections on the envelope known as spikes.	

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8.14	Virion	The complete infective virus particle is called a virion.	The virion may be identical to the nucleocapsid or it may include a nucleocapsid plus the envelope.	
8.15	Size	Viruses range from 20 to 250 nanometers in size. A nanometer (nm) is 10^{-3} micrometers (μm).	Viral size may be determined by filtration through a membrane filter of known pore size, by ultracentrifugation, or by use of electron micrographs in which the virus is compared with a particle of known size.	A diagram comparing viral size with that of other microorganisms would help in appreciating virus size.
8.16	Symmetry of the Capsid			
8.161	Helical Symmetry	The capsid of some viruses is coiled like a tightly wound rope.	The influenza virus has a helical capsid.	
8.162	Cubic Symmetry	Cubic symmetry occurs in viruses which have morphological units arranged in various regular patterns.	The cubic symmetry of animal viruses is icosahedral (20 triangular faces).	
8.163	Complex Symmetry	Certain viruses have a combination of symmetry types called complex symmetry.	The poxviruses show complex symmetry.	
8.2	Host Specificity	Viruses are capable of invading plant, bacterial, or animal cells. Some viruses can penetrate and replicate in only one type of host cell, whereas others may infect a variety of cells within the particular host.	Host specificity is reflected in part by the fact that animal and bacterial viruses must interact with a specific receptor site on the host cell surface to invade the cell.	
8.3	Bacteriophage	Viruses which invade bacteria are called bacteriophage (phage). A given bacteriophage infects only one or a few strains of a given species. The specificity of the phage for its particular bacterial host cell may be used in identification of the bacterial species. This is called bacteriophage typing.	Bacteriophage are the most easily studied of the viruses since their hosts are bacteria. Coagulase-positive staphylococci may be subdivided by bacteriophage typing.	
8.4	Reactions to Chemical and Physical Agents	In general, viruses are susceptible to the same antiseptics and disinfectants as are bacteria. Viruses are not susceptible to antibiotics. Enveloped viruses are made noninfective by treatment with ether, whereas naked viruses are not (Medical Microbiology, Subtopic 12.4).	Some purine and pyrimidine analogs have been used to treat herpesvirus infections of the eye.	

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8.5	Attenuation of Viruses	By growing viruses under varying cultural conditions, mutants which are capable of producing immunity but are no longer capable of producing disease may be selected. These viruses are said to be attenuated.	Live attenuated virus vaccines produce better immunity than killed virus vaccines because the viruses actually continue to replicate in the cells of the host.	
8.6	Classification	Viruses have been classified according to: their physiochemical properties (type of nucleic acid, symmetry [enveloped or naked], and size); the type of symptoms produced (respiratory, intestinal); and other methods of classification.	Other criteria used in classification of viruses include susceptibility to physical and chemical agents, especially ether, immunological properties, natural methods of transmission, and host tissue and cell tropism.	Diagrams of viral classification schemes may be shown.
8.7	Replication	All viruses require actively metabolizing cells in which to replicate. The host cells provide the necessary enzymes, small molecules, and energy for viral synthesis.		Diagramatic sketches of various replicative cycles of viruses may be studied.
8.71	Adsorption, Penetration, and Uncoating	Animal viruses adsorb to specific host cells and are engulfed into the cells by phagocytosis (viropexia). Once inside the cell the nucleic acid is released from the capsid (uncoating).	Viruses attach to host cells by means of specific attachment sites on the host cell surface. If the host cell does not have the specific receptor sites it cannot be infected by that particular virus.	
8.72	Production of Viral Components	Virus nucleic acid and virus components are produced by the host cell by using information provided by the viral nucleic acid.	During the production of virus nucleic acids and virus components, the complete virus cannot be identified in the cell.	
8.73	Maturation	Maturation of virus particles occurs when the nucleic acid is packaged within the capsid.	On maturation, completed virus particles may be identified within the cell.	
8.74	Release of Virions			
8.741	Cell Lysis	Some types of viruses cause cell death and lysis of the cell with release of mature naked virions.	It is this cell destruction which causes the symptoms of the virus disease.	
8.742	Budding	Some viruses are expelled from the host cell one at a time by a type of reverse phagocytosis. The host cell is not disrupted. The virus buds through the plasma membrane which has been altered to incorporate viral protein. This budding process results in the virus becoming enveloped.	The infected host cell may die or may not be affected at all. The unaffected cell may divide and the virus can be passed on to the daughter cells. Membranes through which the virus is extruded may be nuclear, endoplasmic reticulum, or cytoplasmic.	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
8.75	Site of Replication	In general, DNA virus replication is initiated within the nucleus of the host cell, whereas RNA viruses replicate in the cytoplasm.	Exceptions are the poxviruses, which are DNA but replicate in the cytoplasm.	
8.76	Inclusion Bodies	During virus multiplication, virus-specific structures called inclusion bodies may be produced. These become large enough to be seen with the light microscope after staining. They may be cytoplasmic, nuclear, or both.	Virus inclusion bodies are considered to be the site of development of viruses or, in some cases, masses of virus particles. Negri-bodies, found in neurons in cases of rabies, are examples of inclusion bodies.	Prepared microscope slides of various types of inclusion bodies may be studied.
8.8	Cultivation	Viruses have no metabolic machinery of their own and therefore must rely on the host cell to reproduce the virus particles.		
8.81	Embryonated Chicken Eggs	For diagnostic purposes, for preparation of vaccines or for research, viruses may be cultured in embryonated eggs. The eggs are inoculated in the yolk sac, the allantoic sac, or into the chicken embryo, depending upon the virus.	After incubation of the egg the presence of virus is suggested by curling or tucking of the embryo, thickening of membranes, increase or decrease of fluid, hemorrhage, or congestion. Specific tests have to be performed to detect the presence of a virus.	A diagram demonstrating egg inoculation may be shown.
8.82	Tissue Culture	A tissue culture consists of individual cells suspended in a rich medium in a test tube or petri dish and supplied with proper gaseous and temperature conditions. In the classical example the cells reproduce until the container is covered with a single layer of cells. These cells may be subcultured to produce multiple single-layered cultures (monolayers).	Antibiotics may be added to the medium to prevent bacterial contamination since the antibiotics will not inhibit the viruses. Presence of virus may be determined by plaque formation or by certain changes in the cells (cytopathogenic effects or CPE).	A projector slide of a tissue culture may be shown.
8.821	Types of Tissue Culture	Tissue cultures may be produced from tissue freshly removed from an animal (primary cell culture). An established cell line is a tissue culture which has mutated so that it can be transferred repeatedly without dying out.	Monkey kidney or human placenta may be used for primary tissue culture. HeLa cells are an example of an established cell line. These cells are available commercially.	
8.83	Experimental Animals	Animals such as mice may be inoculated successfully with certain viruses of human origin.	Suckling animals are particularly susceptible to virus infection. The presence of virus may be noted by signs produced.	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
8.84	Cultivation of Bacteriophage	Bacteriophage may spread on medium in a petri dish which has been inoculated heavily with a bacterial suspension. After incubation, the bacterial growth will show areas (plaques) where the virus particles invaded the bacteria and caused cell lysis.	Bacteriophage which is specific for *E. coli* may be isolated from sewage or other natural sources.	Students may heavily inoculate phage agar with *E. coli*. The bacteriophage T4 may be added to liquefied phage agar, which is then added to the petri dish as an overlay. After overnight incubation, the plaques (areas where the *E. coli* were destroyed) may be observed.
9.0	**General Characteristics of Protozoa and Helminths**			
9.1	Protozoa	Protozoa are unicellular non-photosynthetic, eucaryotic organisms. They lack a cell wall and generally are motile.	Some protozoa live as saprophytes; others are parasitic for all or part of their lives.	Live protozoa may be purchased from biological supply houses or found in ponds or streams.
9.2	Trophozoite	Trophozoites (active vegetative stage) are susceptible to dehydration, acid, and various other unfavorable environmental conditions.		Prepared microscope slides of trophozoites and cysts of a variety of species may be studied.
9.3	Cyst	The vegetative or trophozoite stage of certain protozoa may undergo nuclear division, store reserve food material, and secrete a resistant wall forming an inactive stage (the cyst).	In many parasitic species the more resistant cyst stage is necessary for survival outside the body during passage from one host to another. This stage also protects the organism from the destructive action of gastric juices after the cyst is swallowed. Therefore it is the infective stage for species which develop cysts.	
9.4	Life Cycle	The life cycle of protozoa may be simple and direct or complex and indirect. A simple and direct life cycle would involve a trophozoite or cyst which is infective for a new host some time after it leaves the human body and does not involve an intermediate host. A complex and indirect life cycle involves one or more intermediate hosts which harbor the immature stage and a final host which harbors the mature stage of the parasites. Depending on the organism, humans may be either the intermediate or the final host.	Human intestinal, vaginal, and urogenital protozoa have a simple life cycle. Tissue and blood protozoa such as *Leishmania* and *Plasmodium* have a complex life cycle. In *Leishmania*, humans are the definitive host (harbor the intracellular stage) and arthropods are the intermediate host (harbor the extracellular stage). In *Plasmodium* (the etiological agent of malaria), mosquitoes are the definitive host (sexual reproduction takes place here) and humans are the intermediate host (only asexual reproduction occurs here).	Charts which depict the life cycles of various types of protozoa, both free living and parasitic, may be studied.
9.5	Locomotion	All protozoa are motile during at least one stage in their life cycle. Protozoa are classi-	Locomotion may be used in obtaining food as well as responding to other stimuli. In	

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	fied, at least in part, on the basis of their locomotor organelles. These include pseudopodia, flagella, cilia, and undulating membranes.	general, parasitic protozoa are less motile than are free-living species.	
9.6 Classification	Protozoa of medical importance may be divided into four groups; Sarcodina, Mastigophora, Ciliata, and Sporozoa.	Infectious diseases caused by these organisms are common in many parts of the world. In areas where the diseases occur, asymptomatic cases are common.	Prepared microscope slides and preserved specimens are available for all four groups.
9.61 Sarcodina	The Sarcodina include the amoebae which move by pseudopodia. A pseudopodium is formed by the amoeba extending part of itself forward and then pulling the rest of the cell up into the extension.	Reproduction is by binary fission or through cyst formation in which two or more nuclear divisions may take place.	
9.62 Mastigophora	In the trophozoite stage, the Mastigophora possess one or more flagella and, in some species, an undulating membrane. The cell membrane is differentiated into a well-defined pellicle which gives a definite shape to the trophozoite.	Reproduction occurs by longitudinal division or by encystment with the formation of small immature forms. Some parasitic forms require alternation of hosts to complete their life cycle.	
9.63 Ciliata	The Ciliata possess cilia (fine hairlike appendages which sweep in unison) during part or all of their life cycle. They possess two nuclei: a vegetative macronucleus and a generative micronucleus.	The Ciliata reproduce by binary fission and by conjugation, the latter taking place every several hundred generations.	
9.64 Sporozoa	Sporozoa are parasitic, generally without visible locomotor organelles, and usually form spores at some stage in their life cycle. Vertebrates and invertebrates serve as hosts.	Reproduction has become highly specialized in these protozoa and involves both sexual and asexual phases.	
9.7 Physiology	Protozoa are heterotrophs requiring large amounts of water. Most are either strict aerobes or facultative anaerobes.	The Mastigophora and most Sporozoa obtain dissolved organic material through their cytoplasmic membranes. Sarcodina obtain nutrients through phagocytosis, whereas the Ciliata have a cytostome which leads to a gullet through which food particles are taken in.	
9.8 Helminths	Helminths are worms that are considered in medical microbiology because the diag-		Projection slides of eggs, larvae, and adults of helminths may be studied. The

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	nosis of these infections depends principally upon the identification by microscopic examination of eggs or larvae found in feces or other excretions. Some species of helminths are free living and others are parasitic.		larger helminth adults may be purchased as preserved specimens. Charts of the life cycles of the various helminths may be studied.
9.81 Aschelminthes (Nematodes or Roundworms)	Nematodes, are elongated, cylindrical, unsegmented worms tapering toward the head and tail. They have separate sexes and a well-developed digestive system. There may be finlike projections of the neck region or the tail which are an aid in diagnosis.	The roundworms are characterized by a cuticle-covered unsegmented body, complete digestive system with mouth and anus, nervous system, excretory system, genital system, and separate sexes. Diagnosis is made by finding eggs or larvae in feces, urine, blood, or tissue.	
9.82 Life Cycles	The life cycles of the intestinal nematodes vary from simple, in which the egg stage is infective within a few hours or weeks after it is passed in excrement, to complex, in which the developmental cycle outside the host involves free-living generations with the ultimate production of an infective stage.	Nematodes with simple life cycles include *Enterobius*, *Ascaris*, and *Trichuris*. Nematodes with complex life cycles include the hookworms *Necator americanus* and *Ancylostoma duodenale*.	
9.83 Platyhelminthes (Flatworms)	In platyhelminthes the digestive tract is absent or, if present, does not have an anus, with wastes regurgitated through the mouth.		
9.831 Cestodes (Tapeworms)	The cestodes or tapeworms consist of a "head" or scolex, a neck, and a series of body segments (proglottids). Cestodes are monoecious (both sexes in the same organism). Worms may reach 20 feet in length.	The scolex is adapted for attachment to the intestinal mucosa. The unsegmented neck is a budding zone that gives rise to the rest of the worm. Each proglottid is capable of producing eggs.	
9.832 Life Cycle	The life cycle of the Cestode is normally indirect and includes alternation of generations between intermediate and definitive hosts.	*Taenia solium* may involve pigs as the intermediate host and humans as the definitive host, whereas *Hymenolepsis nana* may or may not have an intermediate host, although the cycle is rarely direct.	
9.84 Trematodes (Flukes)	Trematodes are elongated and leaf-shaped parasites usually having two suckers found on the ventral surface.	They often have a mouth surrounded by a muscular sucker and a second sucker for attachment. Most trematodes are monoecious, but the schistosomes (blood flukes) have separate sexes.	

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9.85	Life Cycle	Trematodes have complicated life cycles involving one or two intermediate hosts (one of which is usually a mollusk) and several developmental stages and generations before becoming infective for the definitive host.	Mollusks involved as intermediate hosts of flukes include many families of snails, both freshwater and terrestrial.	
10.0	**Inhibition and Killing of Microorganisms by Physical Agents**			
10.1	Heat	Heat is an effective means of sterilizing many solids and liquids. Sterile is defined as absence of viable organisms. Spores are more resistant to heat than are vegetative bacteria. Moist heat is more efficient than dry heat.	The temperature that kills a 24-hour liquid culture of bacteria at pH 7 in 10 minutes is called the thermal death point. The time required to kill all bacteria in a given suspension at a given temperature is the thermal death time.	
10.11	Moist Heat			
10.111	Boiling	Boiling for 10 minutes will kill vegetative pathogens, but not necessarily endospores of pathogenic organisms.		
10.112	Steam Under Pressure (Autoclaving)	Autoclaving is the most effective practical method for heat sterilization. Exposure to steam (121°C) in an autoclave at 15 lb of pressure for 20 minutes will kill all forms of life including endospores.	Heat-stable media for bacterial cultivation are usually sterilized by autoclaving, and cultures growing on media are autoclaved before being discarded.	Pictures of autoclaves (large floor models and table models) may be studied. Use of the autoclave, if available, may be demonstrated.
10.113	Pasteurization	Pasteurization destroys vegetative pathogens but does not destroy all bacteria nor does it destroy endospores. Foods commonly pasteurized to destroy pathogens include milk and cheese.	The pasteurization of milk involves heating at 60°C for 30 minutes or 72°C for 15 seconds (flash method). The heat of pasteurization reduces the number of bacteria to levels making disease transmission improbable. Some toxins, however, may survive pasteurization temperatures.	
10.12	Dry heat	Glassware, metal, and petrolatum oils are usually sterilized in a dry heat oven at 160 to 180°C for 2 hours. This procedure kills vegetative organisms and endospores.	Dry heat destroys microorganisms by oxidation, whereas moist heat destroys microorganisms by protein denaturation.	
10.13	Incineration	Incineration (burning) kills all microor-	Carcasses of infected animals, infected	

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	ganisms. Inoculation loops for transfer of microorganisms are sterilized by incineration.	dressings, etc., should be incinerated. Incineration of inoculation loops containing organisms with a Bunsen burner may cause sputtering and allow some of the microorganisms to escape before they are killed.	
10.2 Drying	Drying in air at room temperature is lethal to many vegetative pathogens but not necessarily to endospores	There is much variation in the lethal effects of drying. Tuberculosis organisms and bacterial endospores are very resistant, whereas the gonococcus is quite sensitive. For this reason, intimate contact is necessary for continued transfer of the gonococcus.	
10.3 Freezing	Freezing can be lethal to many bacteria although generally it is bacteriostatic (inhibits growth).	Repeated freezing and thawing is an effective way of killing many microorganisms. Freezing and then removing the moisture under negative pressure is a mechanism used for preserving organisms and is known as lyophilization.	
10.4 Ultraviolet Radiation (UV Light)	UV light is often used for reducing the microbial population in operating rooms and laboratories. The effectiveness of UV light is limited by its low penetrability.	UV light acts by forming pyrimidine (thymine) dimers from adjacent monomers on the same DNA strand. In media it also produces peroxides. Both of these mechanisms are harmful to microorganisms and, depending on concentrations, may be lethal. UV light is also harmful to the eyes and skin.	The effects of varying exposures of UV light on the growth of bacteria on nutrient agar may be shown.
10.5 Filtration	Heat-sensitive substances such as toxins, carbohydrates, serum, and plasma are sterilized by being passed through filters which hold back bacteria. Viruses are not retained by bacterial filters.	Cellulose acetate or cellulose nitrate membranes with predetermined pore sizes are most commonly used for filtration.	Pictures or examples of various filtration devices may be shown or demonstrated.
10.6 Physical Disintegration	Grinding bacterial cells will destroy them. High-frequency ultrasonic waves will also disrupt bacterial cells.	It is difficult to attain 100% kill by these methods although ultrasound is more efficient than grinding.	
11.0 Inhibition and Killing of Microorganisms by Chemical Agents			
11.1 Sterilization	Sterilization is a process which kills all	Sterilization of materials is important in	

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	forms of life in a given preparation or environment.	operating and delivery rooms. Gowns, instruments, etc., must be sterile. In the laboratory all glassware and media must be sterile before microorganisms can be isolated and identified.	
11.2 Disinfection	Disinfection is the destruction of pathogens on inanimate objects. It includes any process which kills all microorganisms which are capable of producing infectious disease (pathogens). The action of disinfectants is affected by time, temperature, acidity, and the susceptibility of the various bacteria. The presence of organic material, however, may interfere with the action of many disinfectants.	Disinfection does not necessarily destroy spores or certain nonpathogenic microorganisms.	A laboratory experiment which exposes bacteria and bacterial spores to disinfectants for various lengths of time and various concentrations may be performed.
11.3 Antiseptics	Antisepsis is defined as destruction of pathogens in wounds or on body surfaces. Antiseptics may inhibit the growth of bacteria without necessarily killing them.		An experiment comparing the growth of bacteria in the presence and absence of commercial antiseptics may be performed. For example, saturate a filter paper disk with an antiseptic, place it on a plate seeded with a bacterial culture, and incubate it overnight.
11.4 Representative Chemicals Used for Disinfection and Sterilization	Chemical agents inhibit or kill microorganisms by disrupting membranes, denaturing proteins, or interfering with nucleic acid synthesis or functions.	The types of microorganisms and particular situations often determine the type of chemical agent used for disinfection or sterilization.	Skin flora may be cultured before and after hand washing with an antiseptic solution to demonstrate a decrease in total bacterial population.
11.41 Halogens (Chlorine, Iodine, Bromine, and Fluorine)	Halogens owe much of their bactericidal action to their oxidizing activity which inactivates enzymes. They are effective against sporulating organisms.	Chlorine is commonly used to destroy pathogens in drinking water. However it also reacts indiscriminately with organic material. Iodine is commonly used in a dilute alcoholic solution (2.5%) as a skin antiseptic.	
11.42 Alcohols	Alcohols disorganize the lipid structure of cell membranes and also denature cellular proteins. They do not kill spores and should not be relied upon for instrument sterilization.	Both 70% denatured ethyl or isopropyl alcohol are commonly used as antiseptics.	
11.43 Phenols (Carbolic Acid) and Related Compounds	Phenols and cresols cause membrane damage with leakage of cell contents and	A 5% solution of phenol kills all vegetative organisms and after a few hours will kill	

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	resultant lysis. They are also bactericidal by inactivating enzymes. Their action is not inhibited by the presence of pus, serum, or other extraneous organic matter.	bacterial spores. Cresol is more effective and less toxic than phenol.	
11.44 Detergents (Wetting Agents or Synthetic Soaps)	Detergents are surface active agents which act by disrupting cell membranes.	There are cationic, anionic, and nonionic detergents. Cationic are most effective. An example of a cationic detergent is a quaternary ammonium compound which is water soluble. Detergents are often used in hospitals.	
11.45 Heavy Metals (Silver, Mercury, Lead)	Metallic ions, especially of the heavy metals, are toxic to bacteria. A solution of silver nitrate is commonly used to guard the eyes of the newborn infant at birth from infection and possible blindness due to *Neisseria gonorrhoeae*.	Silver ions combine with and inactivate enzymes. Mercury reacts with the SH groups of proteins and inactivates bacterial enzymes. Organic materials reduce the efficiency of heavy metals.	
11.46 Ethylene Oxide	Ethylene oxide is a gas which is strongly bactericidal and virucidal provided the humidity is 20 to 40%. It readily penetrates polyethylene wrapping material and is used for sterilizing heat-sensitive plastics, e.g., syringes, pipettes, needles, and petri dishes.	Ethylene oxide is very inflammable and concentrations of the gas in the air as low as 3% make an explosive mixture. Ethylene oxide is made nonexplosive by combining it with 90% carbon dioxide. It is extensively used for disinfection of surgical instruments and materials in specially designed autoclaves.	
11.47 Formalin Vapor		Formalin vapor at a concentration of 1 or 2%, is bactericidal and may be used to disinfect rooms, blankets, and bedding. It is efficient if the humidity is over 50% and the temperature is raised to about 50°C. It is also used to disinfect breeding facilities for poultry and other livestock.	
12.0 Antimicrobial Agents			
12.1 Antibacterial Agents	Antimicrobial agents are chemicals which are used for the treatment and control of infectious diseases. Desirable properties of antimicrobial agents include: (i) selective action against the microorganism with minimal damage to the host cells; (ii) minimum side effects; (iii) stability within the host;	The bases of selective toxicity depend upon unique structural features or metabolic processes of the microorganism against which the antimicrobial agent is directed.	

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	(iv) minimal development of bacterial resistance to them.		
12.11 Mechanisms of Action	Antimicrobial drugs act by inhibiting cell wall or protein synthesis, injuring the cell membrane, interfering with nucleic acid synthesis, or interfering with intermediary metabolism.		
12.12 Antibiotics	Antibiotics are chemicals which are produced by bacteria and fungi and are able to inhibit the growth of (bacteriostatic) or destroy (bactericidal) other microorganisms. The chemical structures of many antibiotics have been determined and a few are now produced synthetically.	Some antibiotics have a narrow spectrum in that they are effective against either gram-positive or gram-negative bacteria, whereas others have a broad spectrum and are effective against both gram-positive and gram-negative organisms.	
12.121 Cell Wall Inhibitors	A number of antibiotics interfere with the cell wall synthesis of bacteria. The penicillins and cephalosporins, the major group of cell wall inhibitors, interfere with the final step in cell wall synthesis. Bacteria usually do not survive without intact cell walls, thus cell wall inhibitors are generally bactericidal.	Antibiotics which interfere with cell wall synthesis at earlier steps in the development of the cell wall include cycloserine and bacitracin. Since animal cells do not have a cell wall, they are not directly affected by these antibiotics.	
12.122 Interference with the Cell Membrane	Certain antibiotics act as surfactants and attach to the cell membrane, thereby disrupting its integrity. The regulatory activity of the membrane is compromised, resulting in bacterial death.	Polymyxins and the antifungal agent amphotericin B are examples of antimicrobial agents which interfere with cell membrane integrity.	
12.123 Protein Synthesis Inhibitors	A large number of antibiotics interfere with protein synthesis. The inhibition of protein synthesis does not result in the death of the organisms and thus this group of antibiotics is generally bacteriostatic. Examples are chloramphenicol, tetracyclines, and erythromycin.	One important group of protein synthesis inhibitors is the aminoglycosides which include such antibiotics as streptomycin and gentamicin. In contrast to the other protein synthesis-inhibiting antibiotics, this group of antibiotics is bactericidal.	
12.124 Nucleic Acid Inhibitors	Because of the universality of nucleic acid synthesis and function among all living forms, compounds which selectively interfere with microbial nucleic acid and not host nucleic acid synthesis and function are dif-		

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		ficult to develop. Thus, only a few antibiotics, such as griseofulvin, an antifungal agent, and nalidixic acid, used in urinary tract infections, are clinically useful.		
12.125	Antimicrobial Drugs that Interfere with Intermediary Metabolism (Antimetabolites)	Several synthetic compounds have been developed which show antimicrobial activity. Since intermediary metabolism is generally similar in all forms of life, many compounds which have antimicrobial activity are also toxic to host cells. Only a few have been found to be selective for bacteria and include the sulfa drugs, a number of antituberculous drugs, and some drugs effective against bacteria causing urinary tract infections.		
12.2	Antifungal Agents	Antifungal agents are usually also toxic to host cells because fungi are metabolically similar to human cells (fungi are eucaryotes).	Griseofulvin is used to treat dermatophytes; amphotericin B is used to treat systemic mycoses such as histoplasmosis and cryptococcosis. Mycostatin is used to treat superficial candidiasis.	
12.3	Antiprotozoan Agents	Arsenicals, antimony compounds, quinines, and other chemical agents are used to treat protozoan infections.	Metronidazole is a common agent used to treat intestinal hepatic amoebiasis and trichamoniasis.	
12.4	Antiviral Agents	Antimicrobial agents are not effective against viruses because viruses are not cells and do not have intrinsic metabolic capability. A few chemical compounds that have limited effectiveness against certain viruses have been developed.	Idoxuridine (IDU) is effective against eye infections caused by herpes simplex virus. Adenine arabinoside is also effective against herpes simplex of the eye and also herpes simplex encephalitis. Amantadine hydrochloride is an effective chemoprophylactic and chemotherapeutic agent against influenza Type A.	
12.5	Susceptibility Versus Resistance of Microorganisms	An organism is said to be susceptible to a given antimicrobial agent if it is either killed or inhibited when exposed to a concentration of the drug known to be attainable in the patient. A resistant organism is not killed or inhibited by attainable concentrations.	The higher level required to destroy or inhibit the resistant organism may be toxic to the host or may be unattainable because of rapid excretion or metabolism by the host.	
12.6	Drug Resistance	Many bacteria develop resistance to an	Resistance may be due to production of	

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	antibacterial agent. Selection of resistant strains may be enhanced as a result of inadequate or prolonged treatment. Resistance is more likely to occur with bacteriostatic agents. Resistance to a given agent means that the microorganism has developed the resistance, not the patient.	enzymes which break down the drugs, a change in membrane permeability which does not allow the drug to enter the cell, an alteration of one or more of the synthetic pathways, or an increase in the production of a competitive substrate.	
12.61 Mechanisms of Microbial Resistance	Bacteria develop resistance to antimicrobial agents either by mutation or by gene transfer involving conjugation or transduction. DNA transferred to a susceptible recipient bacterium contains genes from a resistant bacterium which code for resistance factors. In this manner, an organism susceptible to one or several agents becomes resistant to these agents.		
12.7 Use of Antimicrobial Drugs			
12.71 Chemotherapy	In chemotherapy or treatment of a disease, antimicrobial agents are used in the minimum dose required to treat a particular infection in a patient. This will vary with the location, distribution, and metabolic activity of the particular organism as well as the adsorption, distribution, and concentration of the agent in the host.	A bloodborne infection would require a different treatment than would an abcess which is walled off from the bloodstream. An immunocompetent individual would receive different treatment from one whose immunocompetence was impaired.	
12.72 Chemoprophylaxis	Chemoprophylaxis is the use of antimicrobial drugs to prevent the establishment of pathogenic microorganisms in the body. It is often used in selective cases after exposure to pathogens or following certain types of surgical procedures. Chemoprophylaxis is usually limited to the action of a specific drug against a specific microorganism. Generally speaking, the use of antimicrobial drugs for chemoprophylaxis is discouraged and used only under certain specific conditions. The indiscriminate use of antibiotics for chemoprophylaxis tends to enhance the selection of resistant organisms.	Chemoprophylaxis might be indicated for an individual with rheumatic fever and heart valve damage who may be given antibiotics before a tooth extraction. This prevents certain oral microorganisms which enter the blood stream from establishing themselves on the damaged heart tissue. Patients undergoing cardiovascular surgery are usually given antibiotics for a few days before and several days after surgery to prevent bacterial implantation on damaged heart tissue.	

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12.73	Choice and Use of Antimicrobial Agents	Most antimicrobial drugs are excreted in the urine and some are metabolized by the liver. Therefore, to maintain a constant blood level, one must give antimicrobials at constant intervals or in a long-acting form. Once begun, the administration of antimicrobials should be continued long enough to eliminate the microorganism and to minimize or prevent the selection of resistant mutants.	Inadequate antibiotic therapy is often more harmful than no antibiotic therapy. An individual who takes half the prescribed dosage for half the time interval is inviting the selection of resistant microorganisms.	
13.0	**Host-Parasite Relationships**			
13.1	Host-Parasite Relationships	Organisms live in various relationships to each other in nature. In the human body, host-parasite relationships may exist in the mouth, the intestinal tract, vagina, skin, and pathological lesions. Several types of relationships exist which account in part for different organisms living together in the same area.		
13.12	Symbiosis	Symbiosis is the coexistence of two or more dissimilar organisms with a certain degree of constancy.		
13.13	Commensalism	Commensalism is a relationship between two types of organisms in which one organism benefits from the association but the other is not affected.	Aerobic and anaerobic microorganisms may live in the same place. The aerobic organism may create a low oxidation-reduction potential which allows the anaerobic organism to grow and multiply.	
13.14	Mutualism	Mutualism is a type of symbiosis in which both organisms are benefited and survival depends upon the relationship.	Termites eat wood but are unable to digest it. Certain flagellates in the termite's intestinal tract digest the wood. The products of digestion are then metabolized by the termite. The flagellates are in turn unable to survive outside the termite.	
13.15	Parasitism	Parasitism is the state in which one organism lives on or within another living organism (the host) in any environment favorable for growth. The parasite benefits at the expense of the host.		

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
13.2	Pathogen	A pathogen is a microorganism capable of causing disease.		
13.3	Normal Flora	The normal flora of the human body include all the microorganisms which live on the skin and mucous membranes of humans without producing disease.	The resident flora consist of those microorganisms which live on or in the body for relatively long periods of time. The transient flora consist of microorganisms that inhabit the body for perhaps hours, days, or weeks but do not establish themselves permanently.	The normal flora of the skin may be cultured and identified in the laboratory.
13.31	Significance of Normal Flora	The normal flora are valuable to the host in keeping the relative numbers of each type of organism in balance and excluding other potentially pathogenic microorganisms.	*Pseudomonas* (a potential pathogen) may be present in the intestinal tract. The numbers of such organisms are kept in check by the normal flora so that clinical disease does not occur.	
13.32	Opportunists	Opportunists are organisms present in the environment or as part of the normal flora of the host which will produce disease under extraordinary circumstances. If in an individual the balance of the normal flora is upset or the host defenses are compromised an opportunistic organism may produce disease.	Alpha-hemolytic streptococci are part of the normal flora of the mouth. After tooth extraction however, these organisms may gain entrance into the bloodstream and produce heart damage.	
13.33	Changes in Body Flora	Antibiotic therapy, natural or therapeutic changes in hormone level, infectious or organic disease, massive X-irradiation, and immunosuppressive agents may cause a change in the number and kind of bacteria present in a given area.	An individual who has had extensive treatment with antibiotics may develop candidiasis. This fungus, which is normally present in low numbers, flourishes after the antibiotic-sensitive members of the normal bacterial flora are removed or diminished.	
13.4	Disease States			
13.41	Disease	Disease is a detrimental change in function or structure in an individual which is discernable clinically. An acute disease has a relatively rapid onset and a short duration, i.e., influenza. Chronic diseases such as tuberculosis have a gradual onset and a long duration.		

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
13.42	Infection	Infection is the invasion and multiplication of microorganisms in tissues with or without the production of disease.	Poliovirus may produce an infection with or without symptoms.	
13.421	Infectious Disease	An infectious disease is one produced by a microorganism.	Influenza, gonorrhea, athlete's foot and malaria are all infectious diseases.	
13.422	Communicable (Contagious) Disease	A communicable disease is one in which the etiological agent is readily transmitted from person to person.	The common cold is a communicable disease. A patient with tetanus is considered to have a noncommunicable disease since it is not spread from person to person.	
13.423	Localized Infection	A localized infection is an infection restricted to one part of a tissue or area of the body.	A boil or a wart on the skin is a localized infection.	
13.424	Generalized Infection	A generalized infection is an infection involving many tissues, organs, or systems of the body.	Typhoid fever is an example of a generalized infection.	
13.425	Inapparent (Subclinical) Infection	An individual who had an inapparent infection does not have clinical symptoms, though specific immunity may be initiated or bolstered. The infectious agent may or may not spread to other susceptible individuals.	Mumps virus may produce an apparent or inapparent infection, both of which produce a long-lasting immunity.	
13.426	Latent Infection	A latent infection is a persistent inapparent infection which has the ability to intermittently produce clinical signs of disease.	Herpesvirus may produce a latent infection. The infected individual may show no clinical signs until after a severe sunburn or a common cold, after which fever blisters due to the viral infection appear.	
13.427	Carrier	A carrier is an individual who harbors a potential pathogen and is capable of spreading it to the environment or to other susceptible individuals. The carrier does not exhibit clinical signs or symptoms of the disease.	Shigella species may be carried in the intestinal tract without producing symptoms in a given individual, but the individual may be able to transmit the organism to other people by means of food or water contaminated with fecal material.	
13.428	Secondary Infection	When one organism invades a host and produces clinical or subclinical disease, a second organism then establishes itself and produces clinical disease on the damaged	An example of a secondary infection is a lesion produced when a person who has chicken pox scratches the pox lesion, allowing dirt and bacteria to reach the damaged	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	tissue. The second organism is usually not capable of initiating an infection alone.	tissue. A purulent lesion (one containing pus) may result as a secondary infection.	
13.5 Host Resistance Factors			
13.51 Mechanical Factors	Intact skin, the mucous coating of the upper respiratory and intestinal tract, and the cilia of the respiratory tract are all examples of mechanical barriers to microorganisms.	Burn patients develop infections rapidly because of the loss of intact skin. Decreased capillary action and the loss of mucous coating of the respiratory tract in heavy cigarette smokers may lead to increased respiratory infections.	
13.52 Chemical Factors	Chemical barriers to disease-producing organisms include gastric juice, complement, lysozyme, beta-lysine, and interferon.	Chemical barriers to disease-producing organisms include: gastric juice, which is extremely acidic; complement, a normal blood protein which is active in certain antigen-antibody reactions as well as in some non-immunological ones; lysozyme, an enzyme which dissolves some bacterial cell walls and is found in tears, nasal secretions, and saliva; and interferon which acts by preventing viral replication in other healthy cells.	
13.53 Individual Factors	People differ as to their susceptibility to infection. This varies with their nutritional state, age, sex, and certain physiochemical factors.	Debilitation, physical stress, hereditary differences, and emotional factors alter an individual's resistance to infection.	
13.54 Nonsusceptibility of the Species	An organism may be pathogenic for one host species, but another host may be completely resistant to infection and/or disease by the same organism.	Non-susceptibility of a host may be temperature related as in the case of anthrax, a bacterial disease of cattle. The chicken is not succeptible to anthrax unless its body temperature is lowered, in which case it then becomes susceptible. In many cases the exact cause of non-susceptibility is not known, e.g., humans get mumps but canines do not.	
13.55 Environmental Factors	Climate, crowding, poor sanitation, smog, and other factors may all act as predisposing factors to infectious disease.	Tuberculosis is an example of a disease which occurs more frequently in crowded conditions such as in institutions and housing developments.	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
13.56	Inflammation	Inflammation is the normal response of a host to injury. It is characterized by an increase in permeability of capillaries in the area which leads to swelling and redness. White blood cells (neutrophils and later macrophages) invade the area and phagocytize cell debris and/or foreign substances.	The inflammatory response to injury may or may not be associated with an infectious process. A sterile blood clot may cause an inflammatory response. On the other hand inflammation caused by a bacterium in the lung (pneumonia) is an infectious inflammatory process. The inflammatory response includes the release of certain compounds which increase the permeability of small blood vessels (capillaries). These compounds are said to be vasoactive.	
13.57	Phagocytosis	Phagocytosis is the engulfment of particles by a polymorphonuclear neutrophil or macrophage.	Phagocytosis is the means by which the body removes dead host cells and debris as well as foreign particles.	A diagram illustrating phagocytosis may be studied.
13.58	Reticuloendothelial System	The reticuloendothelial system (RES) includes the mononuclear phagocytic cells of the body found primarily in the liver, spleen, lymph nodes, and bone marrow. These cells help to protect the body by phagocytizing foreign substances and so are important in helping to rid the body of microorganisms.	The spleen, liver, and lymph nodes are especially important in filtering microorganisms, dead cells, and debris from the blood and lymph. Intracellular fluid is returned to the bloodstream by means of the lymphatic system. The unidirectional transport of lymph follows a route passing through a series of tissues by means of lymphatic vessels which resemble veins. These tissues include lymph nodes and lymph nodules which filter lymph and add cells such as lymphocytes to it.	
13.59	Specific Immunity	Specific immunity is directed toward a specific antigen such as a microorganism or its product and is dependent upon the immune system.		
13.6	Factors that Enable Microorganisms to Cause Disease			
13.61	Pathogenicity	Pathogenicity is the ability of an organism to produce disease. It may involve penetration of the host by the parasite or by its toxic products.	*Mycobacterium tuberculosis* and *Staphylococcus aureus* are considered pathogenic bacteria.	
13.62	Virulence	Virulence is the degree of pathogenicity of	Some strains of *M. tuberculosis* and *S.*	The virulence of an organism may be

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	a given strain of a pathogenic organism. Many authors use the words pathogenicity and virulence synonomously. The virulence of an organism may be due to toxigenicity, invasiveness, or other unknown factors.	*aureus* are avirulent. That is, these particular strains are not capable of producing disease. A pathogen may be virulent for one host and not for another.	tested by injecting it into an appropriate animal. For example, virulence of the pneumococcus may be tested by injecting it into a mouse.
13.63 Microbial Toxins	Microbial toxins are poisonous substances produced by a microorganism.	Toxins interfere with the normal activity of a host cell.	
13.631 Exotoxins	Exotoxins are poisonous proteins excreted by certain gram-positive and a few gram-negative bacteria. They are released into their immediate environment which may be the circulatory system or tissue of the host.	Tetanus, botulism, and gas gangrene are examples of diseases caused by exotoxins. Some bacteria produce exotoxins only after lysogenic conversion.	
13.632 Enterotoxins	Enterotoxins are exotoxins which act on the intestinal tract of the host.	Enterotoxins may be under the genetic control of a transmissible plasmid, e.g., *E. coli* enterotoxin.	
13.633 Endotoxins	Endotoxins are part of the cell wall of gram-negative bacteria and are liberated upon cell lysis or death.	All endotoxins produce the same general symptoms in the host regardless of the species of the organism from which they originate.	
13.634 Mycotoxins	Many fungi produce toxins that cause acute or chronic intoxication or cell damage. Some toxins are able to produce neoplasia (tumors).	Aflatoxins from *Aspergillus flavus* may contaminate animal feed or human food and produce severe disease.	
13.64 Invasiveness	Invasiveness is the ability of an organism to spread within a host.	Invasiveness involves counteracting normal host defenses or having attributes which help the organism spread within the host from the original site of the infection.	
13.65 Inhibition of Phagocytosis	Microorganisms may decrease engulfment (phagocytosis) by leukocytes or macrophages by producing toxins which destroy these cells (leukocidin) or by producing capsules which make phagocytosis difficult.	Encapsulated pneumococci or cryptococci are virulent. These same strains without their capsules are avirulent and more amenable to phagocytosis.	
13.66 Survival Within Phagocytic Cells	Some microorganisms may be virulent because they survive or multiply within phagocytic cells and are resistant to the phagocyte's hydrolytic enzymes.	*Mycobacterium tuberculosis* is capable of multiplying within phagocytic cells. In addition, the infection may be spread from one part of the body to another by these parasitized phagocytic cells.	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
13.67	Extracellular Products	Certain bacteria produce enzymes which are involved in the infectious process.	Coagulase produces clot formation around a lesion and is produced by *Staphylococcus aureus*. Leukocidin, a substance which destroys leukocytes is also produced by *S. aureus*. Hyaluronidase, an enzyme which breaks down the ground substance in connective tissue, is produced by certain streptococci. Collagenase, a proteolytic enzyme which promotes the spread of organisms through tissue, is produced by *Clostridium perfringes*.	
14.0	**Immunology**			
14.1	Immunity	Immunity is the ability of an individual to resist and/or overcome a disease to which most or many of its species are susceptible. Immunity is security against any particular disease, infection, or toxic agent.		
14.12	Basis of Immunity	Immunity depends upon specifically reactive leukocytes called lymphocytes. The human body contains approximately 10^{12} lymphocytes which comprise about 1% of total body weight. Most lymphocytes are found in the spleen, lymph nodes, and Peyer's patches.		A diagram of the human lymphoid system and a microscope slide containing stained lymphocytes may be studied.
14.13	The Immune Response	The immune response is a specific adaptive response to a foreign material such as an infectious agent. There are two types of immunity—cellular and humoral—which may aid in eliminating foreign material or rendering it harmless.	If an individual has had measles as a child, he will not get ill on subsequent exposure. He is immune. However, he may still be susceptible to other diseases to which he has never been exposed.	
14.14	Stem Cell Differentiation	A bone marrow stem cell is an undifferentiated precursor cell. Certain stem cells differentiate into lymphocytes. Those which are responsible for humoral immunity (antibody) are called bone marrow lymphocytes or B cells. Lymphocytes which are influenced by the thymus are responsible for cell-mediated immunity and are called T cells.	Stem cells may differentiate into cells of the erythrocyte series, leukocyte series, including granulocytes (neutrophiles, basophiles, and eosinophiles) and agranulocytes (monocytes and lymphocytes).	A diagram depicting the origin of T and B cells and their functional interactions may be studied.
14.2	Antigen (Immunogen)	An antigen is a substance which can in-	The immunizing effect of certain antigens	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		duce a detectable immune response when introduced into the tissues of an animal.	may be enhanced by mixing the antigen with insoluble materials (adjuvant) which keep the antigen in the tissues for long periods of time.	
14.21	Foreign to the Body	Antigens are perceived as foreign to a host's immune system. The host is normally tolerant (nonreactive) to its own tissues and does not consider them as foreign.		
14.22	Nature of Antigens	The best antigens are large, chemically complex macramolecules such as proteins and certain polysaccharides. Antigenicity however, not only depends on the molecule, but on the host system and the specific conditions under which the molecule is introduced to the host.	The term antigen is sometimes used loosely to refer to viruses, whole bacteria and other organisms and cells used to stimulate an immune response. These materials actually may contain hundreds of different antigens.	
14.23	Antigenic Determinants	Antigenic determinants are those parts of the antigen molecule that are actually involved in binding with antibody.	Antigenic determinants may be as few as three amino acids of a particular protein. A single protein molecule may possess many different antigenic determinants.	
14.24	Haptens	A hapten is generally a small molecule which is incapable of initiating an immune response unless it is linked to a large carrier molecule.	Some individuals are sensitive to their metal watchbands. The metal, as a small molecule or hapten, must combine with the protein of the individual's skin to act as a carrier before the hapten can become antigenic.	
14.3	Humoral Immunity	Humoral immunity results from the stimulation of B cells and their transformation into plasma cells which secrete immunoglobulins.	Humoral immunity is especially important in protection from bacterial and viral disease. The types of infections combatted by B cells was determined partially by studying children born without B cells.	
14.31	B Lymphocytes (Cells)	B cells are located in germinal centers within follicular areas of the spleen and lymph nodes, and a small number circulate through the lymphatics and bloodstream. The presence of immunoglobulins on their cell surfaces differentiate B cells from T cells. About 8 to 10% of circulating lymphocytes are B cells.		

	TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
14.32	Sensitization of B Lymphocytes	B lymphocytes are able to recognize foreign material (antigens) either directly or with the help of T lymphocytes or macrophages. They undergo changes as a result of the reaction and are said to be sensitized. The sensitized B lymphocytes differentiate into clones of memory cells for future response and into antibody-synthesizing plasma cells.	Macrophages are large mononuclear phagocytic cells which appear to be involved with processing certain antigens before they are presented to the lymphocytes.	
14.33	Immunoglobulins (Antibody)	Antibodies are immunoglobulins. Immunoglobulins are a specific family of heterogeneous proteins which are formed in response to antigenic stimulation and react specifically with the antigen that induced their production. They are present in the humors, or fluids of the body such as blood, lymph, and tissue fluids.	Older terminology for immunoglobulins includes gammaglobulins since these proteins migrate primarily in the gamma area upon electrophoresis of serum.	
14.34	Structure of Immunoglobulins	Immunoglobulins are composed of one or more basic units or monomers. Each monomer contains two pairs of polypeptide chains; one pair is approximately twice the molecular weight of the other pair. The chains of high molecular weight are always identical and are called heavy or H chains. The lower molecular weight chains are also identical with each other and are termed light or L chains.	The polypeptide chains are joined together by disulfide bridges.	A diagram depicting the structure of the basic immunoglobulin unit may be studied.
14.35	Classes of Immunoglobulins	There are five antigenically different H chains designated as alpha, gamma, mu, delta, and epsilon. They are the basis for the five classes of immunoglobulins: IgA, IgG, IgM, IgD, and IgE. There are two antigenically different L chains designated as kappa and lambda. L chains are not distinct for any class of immunoglobulin, however, on any given immunoglobulin molecule both L chains are either kappa or lambda.	Most classes of immunoglobulins have been further antigenically divided into subclasses.	Charts listing the properties of the five immunoglobulin classes may be studied.
14.36	V and C Regions	Each immunoglobulin chain contains an amino terminal portion called the variable		

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	or V region and a carboxy terminal portion called the constant or C region.		
14.361 V Region	The V region is very heterogeneous and contains the portion of the molecule which combines with the antigen. The antigen binding site of the immunoglobulin molecule is formed by small numbers of amino acid in the V region of the H and L chains.	Any given antibody molecule is specific for a given antigen and does not react with other antigens. The heterogeneity of the antigen binding site enables immunoglobulins to be specific for any given antigen. A given plasma cell is committed to making antibody of only one specificity.	
14.362 C Region	The C region is fairly constant as to amino acids and is therefore used to antigenically classify the H chains into the five immunoglobulin classes and the L chains into kappa and lambda. The C region of the H chains is that portion of the antibody molecule which binds complement after the molecule has combined with its corresponding antigen (Medical Microbiology, Subtopic 14.38).		
14.363 IgG	IgG is the major immunoglobulin (80%) in the serum of humans and is extremely important in providing protection from reinfection. It is the only immunoglobulin to freely pass the placental wall and is therefore important in providing immunity to the newborn.		
14.364 IgA	IgA occurs in serum but it is most important in mucous secretions. Two molecules of IgA are joined by a secretory piece and are called secretory IgA. This antibody is a major defense mechanism due to its abundunce in saliva, tears, colostrum and other secretions.	IgA aids in preventing an antigen from invading the body and establishing infection.	
14.365 IgM	IgM is the largest immunoglobulin and exists as pentamer with a molecular weight of approximately 900,000. It is predominant in early immune responses and is especially important in protection against infections caused by gram-negative bacteria.	IgM efficiently agglutinates cells. Transfusion reactions between blood types A, B, O, and AB are a result of IgM antibodies to the different types of erythrocytes. IgM is formed actively by the fetus and reaches adult levels in the serum by 1 year after birth.	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
14.366	IgD	IgD is normally present in serum in trace amounts. Its specific functions have yet to be established; however, it is the predominant immunoglobulin on the surface of human B lymphocytes.		
14.367	IgE	IgE is present in even smaller amounts than IgD. These sensitizing antibodies are responsible for type I hypersensitivity or allergy such as hayfever. They exist in high concentrations in allergic individuals and are bound to mast cells and certain leukocytes. Upon reaction with a specific antigen (allergen) they trigger the release of certain substances, e.g., histamine, which are responsible for the signs and symptoms of immediate hypersensitivity.		
14.37	Mechanism of Action of Antibodies	Antibodies interfere with or inhibit the activities of foreign agents through mechanisms such as: increased opsonization which facilitates phagocytosis of microorganisms and other materials; cytolysis of certain microorganisms and other cells in the presence of complement; neutralization of toxins; and prevention of the adherence of microorganisms such as viruses to their target host cells.		
14.38	Complement	Complement is a system of serum proteins which is activated by certain antigen-antibody reactions and is necessary for antibody-mediated cell lysis. When antigen and antibody react in vitro, complement is also consumed. This is used as the basis of the complement-fixation test which may be used to detect the presence of antigen or antibody.	Although complement consists of many proteins, it is referred to as if it were one substance. An alternate pathway in which antigen-antibody reaction is not necessary is also known to activate complement. Complement is also important in many other phases of inflammation.	
14.4	Cell-Mediated Immunity	Cell-mediated immunity is mediated by T cells and is dependent on the presence of the thymus at birth. It is responsible for allograft rejections and delayed hypersensitivity re-	Organ transplants (e.g., kidney, heart) are rejected because the antigens of one individual may be different from those of another. Lymphocytes of a recipient react with the	Diagrams and projection slides showing the architecture of the thymus may be studied.

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	actions and is important in recovery from viral infections.	histocompatibility antigens of the donor organ and cause rejection of the graft if the donor and recipient are not compatible.	
14.41 T Lymphocytes (Cells)	T cells are lymphocytes which have been influenced by the thymus. They vary greatly in the chemical nature of their surface structure and are therefore capable of reacting with different antigens and causing a variety of effects. They do not produce antibodies. Each T cell will react only with the particular antigen for which it has the appropriate receptor sites on its surface. Some T cells assist other cell types such as B cells and macrophages by producing molecules that regulate cell function. These T cells are either helper or suppressor cells.	T cells are found in the subcortical region of lymph nodes and are the only type of lymphocytes found in the thymus. Both T and B cells may be stimulated by the same molecule. T and B cells occupy different areas within the same lymphoid tissue.	
14.42 Sensitization of T Cells	A T cell which comes in contact with an antigen for which it is specific is said to be committed or sensitized. Sensitized T cells multiply after exposure to antigen and give rise to clones of cells.		
14.43 Lymphokines	When a sensitized T cell comes in contact with its corresponding antigen it may release soluble factors (lymphokines) which play a role in cell-mediated immunity. Lymphokines are active in localizing an infection or tumor, in increasing the inflammatory response, and, ultimately, in degrading an antigen.	There are many lymphokines. A few of the more investigated include: macrophage inhibition factor (MIF), lymphotoxin, chemotactic factor, and interferon.	
14.5 Acquired Immunity	Immunity attained as a result of exposure to antigen is said to be acquired. Immunity to a specific antigen may be actively or passively acquired.		
14.51 Acquired Active Immunity	Active immunity may be acquired naturally by having an infection or artificially by an immunization procedure such as vaccination.		
14.511 Primary Response	The immune response that occurs after		

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		initial exposure to a given antigen is called the primary response. With humoral immunity, IgM is usually the first antibody produced and is detectable in about a week.		
14.512	Secondary Response	Upon reexposure to the same antigen, the body produces a greater response in a shorter period of time due to the increased amount of memory lymphocytes formed after the primary contact. The humoral secondary or anamnestic response produces IgG, and these antibodies may be detectable for long periods of time providing long-lasting protection.	Two or more injections of the same antigen spaced over a period of time are given in immunization programs to produce a long-lasting immunity. Tests to determine whether an individual has specific antibodies to an antigen are performed to determine past or present experience with a specific agent (Medical Microbiology, Topic 20).	
14.513	Immunization (Vaccination)	Vaccination is the injection of substances which stimulate an immune response that protects the host against an infectious disease. The term vaccination was originally used to indicate immunization against vaccinia or cowpox virus which conferred protection to smallpox virus. The injected agent is called a vaccine.	The exact timing of an immunization sequence depends upon the type of vaccine used, the route of inoculation, and the immunological competence of the individual. Vaccines are not always injected, e.g., the Sabin vaccine is taken orally.	
14.514	Types of Vaccines	Products used as vaccines include: killed microorganisms; attenuated microorganisms, i.e., organisms which are living but which have been altered so as to produce immunity but not disease; and toxoids, i.e., toxins which have been altered so as to produce immunity but not disease.	The Salk polio vaccine contains killed poliovirus, whereas the Sabin polio vaccine consists of live attenuated poliovirus. Immunization to tetanus and diphtheria is carried out with tetanus and diphtheria toxoids. DPT vaccine refers to diphtheria, pertussus, and tetanus and is given to infants at 2 months of age with boosters at various time intervals.	
14.52	Acquired Passive Immunity	In passively acquired immunity, the immune response is not produced by the host. Preformed antibodies may be acquired naturally from the mother by the unborn child across the placenta, or in the colostrum after birth. Specific antibodies may also be obtained in the form of gammaglobulin or hyperimmune serum which is administered by injection.	A baby will receive passively acquired immunity to antigens for which the mother has circulating IgG antibodies. Tetanus is an example of a disease treated by immunoglobulin that has specific antibodies to the tetanus toxin which have been formed in another individual.	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
14.6	Immune Mechanisms in Tissue Damage	Protective immune responses to an infectious agent may also produce significant pathological effects in the host. In fact, the inducing agent, if any, for many deleterious effects of the immune system is unknown. Whether or not the immune response is beneficial or deleterious, the resultant tissue damage is classified into four basic types: anaphylactic, cytotoxic, immune complex, and delayed hypersensitivity.		
14.61	Type I Anaphylaxis or Immediate Hypersensitivity	Hypersensitivity is the immune response gone awry, resulting in irritation and/or tissue damage. Immediate hypersensitivity is mediated by IgE and is manifest by reactions occurring within minutes after antigen combines with antibody. The reaction may occur in any member of a species and is called anaphylaxis. Atopy is also mediated by IgE. It is the hereditary tendency to develop immediate hypersensitivity states such as hay fever and asthma to allergens such as pollens and insect venoms that do not induce a response in normal individuals.	IgE antibodies are also called reagin or homocytotropic antibodies because they bind to mast cells and cause the release of vasoactive substances such as histamine when they combine with antigen. Anaphylaxis may be generalized, resulting in shock and possibly death. Localized anaphylaxis may occur in target organs such as the skin, nasal mucosa, or gastrointestinal tract. People who are allergic to a specific substance, e.g., penicillin, must receive at least one sensitizing dose. Reexposure to the same substance may then elicit the anaphylactic reaction. Allergy densensitizing treatment consists of small doses of the offending allergen (antigen), which stimulate the production of blocking antibodies in the serum. These IgG antibodies act to block or neutralize the antigen so that it does not have an opportunity to come in contact with the cell membrane-attached IgE.	
14.62	Type II Cytotoxic Reactions	Cytotoxic reactions involve IgG or IgM antibodies which bind to antigens on cell membranes. Complement is then activated, resulting in cell lysis.	The target for the cytotoxic reaction may be either a formed blood element (red cells, white cells, platelets) or a specific cell type within a particular tissue.	
14.63	Type III Immune Complex Reactions	These reactions are a result of IgG or IgM immune complexes (antigen-antibody) occurring in tissue with resultant inflammation. Serum sickness is a classic example in	Treatment of diphtheria and tetanus with horse antiserum to the toxins of these organisms has resulted in many patients developing antibodies to the horse serum proteins	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	which signs and symptoms develop about 2 weeks after injection of foreign serum. Antigen-antibody complexes form in the bloodstream and are deposited at different sites in the body.	with resultant serum sickness. The use of human serum for treatment of these diseases has essentially eliminated this secondary side effect.	
14.64 Type IV Delayed Hypersensitivity	Delayed hypersensitivity reactions are cell mediated and occur as the result of the interactions between sensitized T cells and their corresponding antigens (Medical Microbiology, Subtopic 14.4). The reaction takes 24 to 72 hours to reach its peak. Poison ivy, detergents, and heavy metals are examples of substances which may induce delayed hypersensitivity. Second and subsequent exposures of the patient to the allergen may result in a reaction. Delayed hypersensitivity skin testing detects cutaneous sensitivity to an antigen. In testing for sensitivity to an infectious agent such as tuberculosis, a positive test does not necessarily imply active infection but merely previous exposure.	In tuberculosis, the response to the organism may lead to host cell destruction and cause cavitation in the lungs, i.e., the immune response is the major cause of tissue damage.	
14.7 Autoimmunity	The body is normally tolerant to its own tissue; autoimmunity reflects a loss of tolerance to self. It is specific humoral or cell-mediated immunity to the body's own tissues. Autoimmune disease is a clinical disorder associated with the immune response to self. Examples of autoimmune diseases include rheumatoid arthritis, systemic lupus erythematosus, and chronic thyroiditis.	There are many theories regarding the methods for induction of autoimmunity involving genetics, physiology, immunology, and infectious agents as factors; however, the exact mechanisms remain unclear.	
14.8 Immunodeficiency	Humoral immunity, cell-mediated immunity, phagocytosis, and complement act independently or in concert with each other in combatting infection and disease. Deficiency of one or more of these systems results in increased susceptibility to infectious agents. Deficiencies may be congenital or acquired with symptomatology related to the degree of deficiency.	The type of infection often indicates the type of immunodeficiency present. Deficiencies of T cells, B cells, or both are the most serious disorders. Functional deficiencies exist in which a cell or component is present but is not performing in an adequate manner.	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
14.9	Lymphocyte and Plasma Cell Disease	Diseases which affect the leukocytes, particularly the lymphocytes and plasma cells, result in immunological abnormalities. In multiple myeloma for example, there is a malignant transformation of a single clone of plasma cells, resulting in the production of an abnormally high level of a monoclonal (one specificity) immunoglobulin. Patients with myeloma often present with recurrent infections since they do not have enough normal immunoglobulins to combat disease.		
15.0	**Medical Bacteriology**			
15.1	Disease-Producing Bacteria	Although myriad numbers of bacteria inhabit the earth, only a comparatively small number of species are capable of causing disease.	Bacteria are ubiquitous, and most species are not involved in disease production. However, most treatable infectious diseases are probably caused by bacteria.	Projection slides, motion pictures, and stained microscope slides of medically important bacteria are available. Culturing and examination of live pathogens are not recommended for the untrained microbiologist as serious disease may occur. In many cases other bacteria which may be used safely in the student laboratory are listed.
15.2	Pyogenic Cocci	Pyogenic cocci cause a variety of infections which involve suppuration (pus production).		
15.21	Gram-Positive Cocci (*Bergey's* #14; refers to the category of Bacteria as given in *Bergey's Manual of Determinative Microbiology*)			
15.211	*Staphylococcus*	Staphylococci are arranged in grapelike clusters and form large opaque gold to white colonies. *S. aureus* is a pathogen which is a transient inhabitant of the anterior nares of an appreciable percentage of the population. It produces many toxins and enzymes including coagulase, leukocidin, and enterotoxin. Staphylococci are the most common cause of wound infections, boils and other	Diseases produced include boils, abscesses, impetigo, pneumonia, meningitis, and septicemia. Staphylococcal infections tend to be more localized than other pyogenic infections such as streptococcal infections. Staphylococci are a common cause of food poisoning due to consumption of preformed enterotoxin in food which has been im-	*S. epidermidis* is recommended for laboratory experiments involving staining, colonial, and biochemical characteristics. Slides of coagulase production by *S. aureus* may be projected.

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	acute pyogenic (pus-producing) infections in humans. *S. aureus* is differentiated from other staphylococci on the basis of its coagulase production. Coagulase-negative staphylococci such as *S. epidermidis* are part of the normal skin flora but may also cause disease such as subacute bacterial endocarditis.	properly handled and contaminated with *S. aureus*. *S. aureus*, especially methicillin-resistant strains, is an important cause of nosocomial infections. Antibiotics can be used to treat staphylococcal disease, but susceptibility testing should be performed due to prevalence of plasmid-associated antibiotic resistance.	
15.212 *Streptococcus* (other than *S. pneumoniae*)	Streptococci are arranged in chains and produce small, translucent colonies on solid media. Streptococci are abundant in nature, and some are often members of the normal flora of the mouth and intestinal tract. The streptococci may be divided into three groups according to their hemolytic activity on blood agar plates. Hemolytic streptococci may be serologically grouped (A to O) based on the C carbohydrate of their cell wall (Lancefield typing).	Major diagnostic tests include determination of hemolysis type (alpha-hemolysis, greening of erythrocytes; beta, clearing of cells; gamma, no change), and serotyping.	Projection slides showing hemolysis may be shown.
15.213 *Streptococcus pyogenes*	*S. pyogenes* (beta-hemolytic, group A), elaborates a wide variety of toxins and enzymes which are believed to be involved in its extreme invasiveness and disease production. The M protein in the cell wall aids in preventing phagocytosis of the organism. The streptococci produce a wide variety of acute, fulminating infections and chronic diseases. The common "strep throat" is the best known of these. Human cases and carriers are the source of infection of *S. pyogenes*.	Diseases produced by *S. pyogenes* include septic sore throat, scarlet fever, endocarditis, urinary tract infections, and wound infections. Important sequelae include rheumatic fever and glomerulonephritis. Group B streptococci are a major cause of meningitis in infants. Alpha-hemolytic streptococci are normal inhabitants of the upper respiratory tract but may cause disease such as subacute bacterial endocarditis. Penicillins are generally used in treatment of streptococcal disease. Enterococci are part of the normal flora of the gastrointestinal tract. They may cause disease when displaced into other body tissues. The enterococci are resistant to penicillin.	Prepared blood agar plates may be purchased. Throat swabs inoculated onto blood agar plates may exhibit hemolytic zones.
15.214 *Streptococcus pneumoniae* (the Pneumococcus)	The pneumococci are lancet-shaped alpha-hemolytic diplococci that sometimes occur in short chains. Virulent strains are en-	*S. pneumoniae* are often differentiated from other alpha-hemolytic streptococci by their susceptibility to optochin.	Projection slides showing susceptibility of this organism to optochin disks may be studied. Films showing resistance of encapsu-

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	capsulated, and over 85 strains have been identified according to capsular antigen. *S. pneumoniae* can be a member of the normal flora of the upper respiratory tract of humans, who then act as carriers. This is the most common agent of bacterial pneumonia, especially in young and elderly patients, and is still a major cause of death in the United States. The virulence of the organism depends upon the presence of the capsule which prevents phagocytosis, thus permitting multiplication of the organism in infected tissue.	A pentavalent capsular vaccine is available for immunization against strains that cause 90 to 95% of U.S. infections. Penicillin is the antibiotic of choice, followed by erythromycin.	lated organisms to phagocytosis are available.
15.22 Gram-Negative Cocci (*Bergey's* #10)			
15.221 *Neisseria*	This genus consists of kidney-shaped diplococci somewhat flattened along adjoining sides. Some species are normal inhabitants of the respiratory tract and occur extracellularly, whereas the human pathogens occur intracellularly.	These organisms are strict aerobes but the pathogenic species grow best in an enhanced CO_2 environment such as a candle jar. The pathogenic *Neisseria* are extremely labile and require special media.	Nasal swabs may be cultured on agar plates and then tested for oxidase-positive colonies demonstrating normal flora such as *Branhamella* (*Neisseria*) *catarrhalis*. A typical candle jar may be demonstrated.
15.222 *Neisseria gonorrhoeae* (the Gonococcus)	*N. gonorrhoeae*, the gonococcus, causes gonorrhea, a highly contagious, sexually transmitted disease. The gonococci attack mucous membranes of the genitourinary tract and produce acute, pus-filled lesions which may result in chronic inflammation. Both women and men may be asymptomatic carriers. The only natural host for *N. gonorrhoeae* is the human. Gonorrhea now occurs in epidemic numbers, with the highest incidence in the 20- to 24-year age group. The infection rate can be reduced by early diagnosis and treatment of cases and sexual contacts and by education.	Gonococcus infection of the eye (ophthalmia neonatorum) is acquired by newborns during passage through an infected birth canal if not prevented by application of silver nitrate onto the eyes immediately after delivery. Penicillin G or ampicillin is the drug of choice in treatment of *N. gonorrhoeae* infections, but penicillin-resistant strains have recently become a problem.	Microscope slides with stained smears from the local venereal disease clinic showing gram-negative intracellular diplococci may be examined in the laboratory.
15.223 *Neisseria meningitidis* (the Meningococcus)	*N. meningitidis* is an obligate parasite of humans and is found as part of the transient flora of the nasopharynx in up to 25% of the population. From the nasopharynx the meningococcus	Microscopic observation of organisms in the cerebrospinal fluid permits presumptive evidence of bacterial meningitis and indicates prompt chemotherapy. Penicillin G is the antibiotic of choice.	

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		may spread to the central nervous system where it causes meningitis. Untreated cases are fatal in 70 to 100% of patients, and death may occur in 24 hours.		
15.3	Rods (*Bergey's* #15)	These organisms are saprophytes or intestinal commensals of warm-blooded animals. The spores may remain viable in soil for years.		
15.31	*Clostridium*	The clostridia are large gram-positive, spore-forming, anaerobic bacilli. They produce powerful exotoxins and enzymes. They are free-living inhabitants of soil. In addition many are normal commensals of the intestinal tracts of humans and animals. These organisms are not highly invasive but may produce disease by elaboration of highly injurious toxins.	Differentiation of species within the genus is by colonial appearance, morphology of spores, and biochemical tests. The antigenic type of toxin is identified by neutralization with specific antiserum.	*C. tetanomorphans* may be grown on plates in anaerobic jars or grown anaerobically in thioglycolate broth. Its morphology is similar to that of *C. tetani.*
15.311	*Clostridium botulinum*	The botulism bacillus produces the most potent neurotoxin known. The toxin acts by blocking transmission of nerve impulses to the muscles. Botulism is an intoxication resulting from ingestion of improperly preserved food in which *C. botulinum* has grown and produced toxin. The organism does not have to be present to cause the disease.	Botulism is a neurological disease, and gastrointestinal symptoms are slight or absent. The mortality rate is high but occurrence of the disease is rare. The toxin can be inactivated by heating to 100°C for 20 minutes. Epidemics of botulism occur when improperly home-canned or commerically canned foods are served without adequate cooking. Infant botulism results from toxin formed by *C. botulinum* present in the intestinal tract. Botulism is treated with specific antitoxin.	Projection slides or films may be shown to illustrate the epidemiological principal of this foodborne disease.
15.312	*Clostridium tetani*	This organism produces round terminal spores which give the organism a characteristic "drumstick" appearance. *C. tetani* is not an invasive organism. When accidentally introduced via a penetrating wound, the spores germinate and the organisms multiply. The bacilli remain localized but produce exotoxin, and tetanus (lockjaw) results. The toxin is believed to travel by way of the motor nerves to the	Clinical diagnosis with bacterial confirmation is difficult because of the few organisms present. This is a preventable disease. Prevention depends on active immunization with toxoid to stimulate antitoxin production. Diphtheria toxoid, killed pertussis organisms, and tetanus toxoid are the three antigens administered in a DPT immunization. A "booster" injection may be given to a previously im-	Projection slides showing the endospores or victims of the disease may be shown.

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		brain and spinal cord. By interfering with synaptic transmission it increases excitability of motor nerves and produces convulsive contractions of voluntary muscles.	munized individual who has received a penetrating wound. Treatment of tetanus is with tetanus antitoxin and penicillin.	
15.313	Gas Gangrene Organisms	The most common species of clostridia which produce the disease gas gangrene are *C. perfringens*, *C. novyi*, and *C. septicum*. In gas gangrene, a mixed infection is the rule. Gas gangrene develops after spores contained in soil are introduced into a deep wound in which necrotic tissue has developed. As the organisms multiply, carbohydrates are fermented and gas is produced which causes distension of tissue and interference with the blood supply. The organisms also secrete necrotizing toxins and hyaluronidase, which favors spread of infection. This kills tissue and results in gangrene (local death of the tissue).	Gangrene is often progressive and may necessitate amputation of a limb. Treatment of gas gangrene involves surgical debridement, antimicrobial drug therapy, and polyvalent antiserum. Prevention includes early and adequate cleaning of the wound. In addition to gas gangrene, *C. perfringens* also produces an enterotoxin which when ingested in food induces profuse diarrhea.	Projection slides showing the pathology of this disease may be shown.
15.32	*Bacillus anthracis*	The anthrax bacillus is a large aerobic gram-positive spore-forming rod. Anthrax is primarily a disease of sheep and cattle but may be transmitted to humans as an occupational disease of hide and wool handlers. In humans, anthrax may produce pulmonary or cutaneous infections which may lead rapidly to septicemia and death.	Virulent strains of the organism produce rough colonies (non-encapsulated organisms) on laboratory media. In the presence of CO_2 or in the body capsules are formed. The virulence of the organism depends on the presence of the polypeptide capsule and possibly the exotoxin which is produced. Diagnosis depends on finding the large bacilli in blood or tissue smears. Spores are not seen. The bacilli may be identified by the fluorescent antibody technique. Penicillin may be used to treat anthrax. Historically this organism was used by Robert Koch in establishing his four postulates of the bacterial etiology of a disease.	*B. subtilis* may be used to demonstrate some of the characteristics of this genus.
15.4	Actinomycetes and Related Organisms (*Bergey's* #17)			
15.41	*Actinomycetales*	These bacteria are gram-positive and may produce branching filaments although they commonly have diphtheroid morphology.	The four medically important genera are *Actinomyces*, *Nocardia*, *Streptomyces*, and *Actinomadura*. These bacteria are related	*Streptomyces griseus* may be used to study some of the characteristics of the *Actinomycetales*.

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	Some are acid-fast and some are anaerobic. The *Actinomycetales* are found in the soil, the air, or may be part of the normal flora of many animals and humans. Infections produced by these organisms are infrequent.	to the corynebacteria and the mycobacteria and superficially resemble the fungi. Some books on medical microbiology discuss diseases produced by the *Actinomycetales* under the fungus diseases. Many antibiotics are produced by *Streptomyces* spp.	
15.411 *Actinomyces israelii*	This organism is non-acid-fast and preferentially anaerobic. It occurs as a normal commensal in the mouth and is considered relatively noninvasive. In tissue it occurs as mycelia surrounded by suppurative inflammatory material which produces "sulfur granules." Human actinomycosis is a chronic suppurative and granulomatous disease occurring in the jaws, lungs, or ileocaecal region. Infection in the head or neck area often follows a tooth extraction or dental surgery. The infection spreads by direct extension through draining tracts.	Actinomycetes deprive host tissue of nutrients, thereby causing tissue and cell necrosis. Identification of *A. israelii* is generally by fluorescent antibody procedures. Treatment consists of prolonged administration of penicillin and surgical drainage of the lesions if necessary.	
15.412 *Nocardia asteroides* and *N. brasiliensis*	These organisms are aerobic and many strains are partially acid-fast. They are found in the soil and are not part of the microbial flora of humans. The nocardiae probably enter the body through the respiratory tract or through breaks in the skin. Nocardiosis is an opportunistic pulmonary disease that may spread to other parts of the body.	The organism has a predilection for the brain, and abscess formation may occur by spread of the organism through the bloodstream. Actinomycetoma may also be caused by these organisms. Treatment is with sulfonamides.	
15.42 *Mycobacterium*	These organisms are acid-fast, strictly aerobic rods which frequently show deeply staining granules and often occur in clumps. The mycobacteria do not stain well with the Gram stain. They occur as saprophytes and pathogens of humans and animals. The mycobacteria are rich in lipids which are concentrated in the cell wall. These lipids are partly responsible for the acid-fastness of the organism, the host's cellular reaction to the tubercle bacillus and the tendency of the organisms to clump.	The organisms are slow growing and require special media. Identification of mycobacteria species is by biochemical characteristics.	*M. smegmatis* may be safely cultured to show the slowly growing waxy colonies and the acid-fast rods which tend to stick together. Prepared microscope slides showing mycobacteria stained with fluorescent dye may be studied.

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15.421 *Mycobacterium tuberculosis* (Tubercle Bacillus)	The tubercle bacilli enter the body through the lungs and are ingested by phagocytes. Intracellular multiplication follows. Progressive damage to lung tissue occurs through a complex series of events. During this time, delayed hypersensitivity develops and the disease may become arrested or progressively spread through the lungs or throughout the body, depending on host factors. Malnutrition, poor sanitary conditions, crowding, and hereditary susceptibility are all factors which predispose the individual to active tuberculosis.	Tuberculosis is typically a wasting disease and was originally called consumption. The lung becomes extensively damaged before death. The human and bovine tubercle bacilli (*M. tuberculosis* and *M. bovis*, respectively) are the most common causes of tuberculosis. There are many other species of mycobacteria which produce disease opportunistically in humans. Prevention and control of cases are of primary importance. These measures include detection by mass survey, treatment of cases, elimination of tuberculosis in cattle, and pasteurization of milk. Detection is by skin test which shows delayed hypersensitivity to a purified protein derivative (PPD) of the organism. This is followed by X-ray examination of skin test-positive individuals. BCG vaccine (an attenuated bovine organism) provides some level of immunity after its inoculation into tuberculin-negative individuals who become tuberculin positive. The vaccine is not commonly used in the United States. Treatment involves isoniazid, ethambutol, rifampin, and streptomycin.	Projection slides of the organism and of patients with tuberculosis may be shown.
15.422 *Mycobacterium leprae*	*M. leprae* is the etiological agent of leprosy, a chronic granulomatous infection largely confined to tropical countries.		
15.43 *Corynebacterium*	These granular gram-positive rods are club shaped and, because of incomplete fission, produce V and Z forms. This genus includes *C. diphtheriae*, the causative agent of diphtheria, and a number of commensal diphtheroid bacilli found in the upper respiratory tract or the skin. *C. diphtheriae* is not invasive and grows primarily in the pharynx where it produces a powerful protein exotoxin which blocks protein synthesis and causes the symptoms of the disease. Only strains of *C. diphtheriae*	Diphtheria is characterized by the presence of a grayish necrotic pseudomembrane in the throat and the production of toxic damage to the heart and central nervous system. Paralysis of the throat, eye muscles, and severe damage to the heart are frequent sequelae. Active immunization with diphtheria toxoid is important in prevention of the disease and is administered routinely in the United States in DPT vaccine. Diagnosis includes demonstration of the specific toxin produced by the isolated organism. Treat-	*Corynebacterium* sp. may be isolated from the throat or *C. xerosis* may be purchased and used in the student laboratory. Each will show pallisades, Y and Z forms, and clubbing of organisms.

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		which have undergone lysogenic conversion produce the exotoxin. Diphtheria is acquired by inhaling bacilli contained in droplets exhaled by infective cases.	ment includes early antitoxin administration and penicillin.	
15.5	Gram-Negative Rods			
15.51	Gram-Negative Facultatively Anaerobic Rods (*Bergey's #8*)			
15.511	*Enterobacteriaceae*	Many of these short bacilli are part of the normal intestinal flora of humans and animals. The pathogenic mechanism of these organisms is uncertain but has been attributed to include endotoxin, which all contain, and to exotoxins, which some produce. There are many genera and species of *Enterobacteriaceae* which are important as opportunistic pathogens; however, only a few examples will be given in this outline.	This bacterial family exhibits diverse metabolic activity. All members ferment glucose. Endotoxin is a potent pyrogen (fever-producing substances). Since antibiotic susceptibilities of the *Enterobacteriaceae* strains vary tremendously, testing should be performed on all isolates.	A wide variety of biochemical tests are performed to identify the *Enterobacteriaceae*. A few of these may be performed in the laboratory as time permits. Many commercial identification systems may be used to identify these organisms.
15.5111	*Escherichia*	*E. coli* is a normal commensal in the intestinal tract, but it may invade opportunistically and cause infection in many organs of the body such as the bladder, appendix, and kidney. Certain strains may produce enterotoxins which cause traveler's diarrhea. Some strains may also produce epidemic diarrhea in babies and infants.	*E. coli* is used as an indicator organism, providing evidence of fecal contamination of water supplies and prepared food. It is the most widely studied bacterium and is employed in recombinant DNA research because its chromosome has been mapped in detail.	Eosin methylene blue (EMB) plates may be inoculated with *E. coli* to show typical colonies and green metallic sheen.
15.5112	*Klebsiella*	*Klebsiella* typically produces a well-defined capsule and profuse mucoid growth on solid media. *K. pneumoniae* causes a destructive type of pneumonia and is a secondary invader of the respiratory tract. This organism occasionally may produce suppurative infections in many parts of the body and may be responsible for infections of the urinary tract.	*K. pneumoniae* is primarily parasitic but may be found as a commensal of the upper respiratory and intestinal tracts in humans.	
15.5113	*Serratia*	The type species is *S. marcescens*, which may produce red pigment on cultivation. It is an opportunist, and nonpigmented strains	*Serratia* strains are found commonly in water and soil. *Serratia* strains are often resistant to many antibiotics.	

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	have been the cause of nosocomial infections. It causes urinary and respiratory tract infections and septicemia.		
15.5114 *Proteus*	The type species is *P. vulgaris*. Its cultural characteristics include a tendency to swarm over the agar surfaces. These opportunistic organisms may cause severe infections of the urinary tract and wounds.	These free-living organisms of soil and water frequently inhabit human intestinal tracts. They are often resistant to many antibiotics.	*P. vulgaris* may be grown on agar plates to show swarming. Motility of the organism may be detected in a wet mount observed under a microscope.
15.5115 *Salmonella*	The salmonellae are parasites of the intestinal tracts of humans and animals, including birds. These organisms are spread in food or water via the fecal-oral route, producing a clinical or subclinical infection. Onset of symptoms coincides with liberation of a large number of organisms into the bloodstream. *S. typhi* produces the most serious disease, followed by *S. enteritidis* and *S. choleraesuis*. Improperly handled raw meat, poultry, shellfish, or cracked eggs may serve as sources of infection. Food handlers or excreta from infected rats or mice may also be sources of infection.	The salmonellae cause three main types of disease, but mixed forms are frequent: enteric or typhoid fever, in which the salmonellae are ingested with food or drink, enter the intestinal tract, pass through the lymphatics to the bloodstream and are distributed to many organs; septicemias, which may lead to local suppuration; gastroenteritis ("food infection"), due to *S. enteritidis* serotype *typhimurium* and other serotypes. During the first 2 to 3 weeks of the enteric fever the patient's blood is cultivated to isolate the organism.	Typing of *S. gallinarium* may be used as a demonstration of serotyping of salmonellae.
15.5116 *Shigella*	Although the shigellae are found in a few primates, humans are thought to be the sole reservoir of infection. The shigellae produce bacillary dysentery, with infection limited almost entirely to the gastrointestinal tract. The most common species are *S. flexneri* and *S. sonnei*. All species are pathogenic by way of endotoxin released upon autolysis. Shigella infections are acquired by the oral route from feces of human cases or carriers.	*S. dysenteriae* causes the most serious disease and produces an exotoxin which has neurotoxic and enterotoxic properties. The resulting bacillary dysentery is an acute inflammatory condition that leads to ulceration of the large intestine. No bacteremia is produced. Diagnosis is by cultivation, with differentiation from the salmonellae. Ampicillin or chloramphenicol may be used for treatment but the disease is usually self-limiting, and only fluid replacement may be necessary.	
15.5117 *Yersinia (Pasteurella) pestis*	*Y. pestis* is a small bacillus showing bipolar staining and causes plague in humans and animals. Some strains are highly virulent. Plague, a disease of rats and other rodents including the ground squirrel, is	There are three clinical types of plague: bubonic, which causes enlargement of regional lymph nodes; pneumonic, which may lead to bronchopneumonia; and septicemic. Prevention includes control of rats and their	Prepared microscope slides showing bipolar staining may be studied. Projection slides of fleas, rodents, and humans with the disease may be shown.

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	transmitted by flea bites from rodent to rodent and from rodent to human.	fleas. The most effective drug is streptomycin. *Y. enterocolitica* is responsible for an increasing number of cases of bacillary gastroenteritis and bacteremia. Swine, dogs, and humans may be the source of infection. *Y. pseudotuberculosis* produces infection in birds, rodents, and other animals that is rarely transmitted to humans. Disease is often chronic and associated with nodules superficially resembling tubercules that occur in the intestine or abdominal organs.	
15.512 *Vibrionaceae*			
15.5121 *Vibrio*	The vibrios are comma-shaped, motile bacteria. The most important species is *V. cholerae*, which causes cholera—a disease endemic in parts of Asia and Africa. Cholera is a violent infection of the intestinal tract causing loss of gallons of water within a day or two. A general invasion of the body does not occur.	In cholera, an enterotoxin is responsible for the outpouring of fluid from the intestinal tract. This potent protein stimulates excessive formation of cyclic adenosine monophosphate, causing the intestinal wall to secrete electrolytes and water into the lumen. The disease is spread by water contaminated by human feces. Effective treatment of cholera is by oral administration of a balanced salt solution containing glucose. *V. parahaemolyticus* causes acute enteritis after ingestion of contaminated seafood.	
15.52 *Bergey's* "Genera of Uncertain Affiliation"			
15.521 *Haemophilus*	This genus consists of tiny coccobacilli which require one or both of the growth factors known as X (hemin from blood) and V (nicotinamide adenine dinucleotide [NAD]). CO_2 enhances growth. Some of these bacilli produce satellitism around *S. aureus* or certain other bacteria which excrete large amounts of NAD. The nonencapsulated form of *H. influenzae* is a part of the normal respiratory flora of humans. The encapsulated form, especially type b, produces suppurative res-	Transmission is from person to person by the respiratory tract. *H. influenzae* was misnamed and does not cause influenza which is a viral disease. Diseases caused by other species include pink eye (*H. aegyptis*) and chancroid, a venereal disease (*H. ducreyi*).	Projection slides of *Haemophilus* spp. showing satellitism and need for X and/or V factors may be shown.

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	piratory tract infections, including middle-ear infections and meningitis in young children. This form of meningitis is highly fatal if untreated.		
15.53 Gram-Negative Aerobic Rods (*Bergey's* #7)	These bacilli are primarily nonenteric opportunists responsible for diverse infections.		
15.531 *Pseudomonas*	The pseudomonads are plant pathogens or free-living organisms of soil and water that may be part of the normal flora of the skin and intestines of humans. An important species is *P. aeruginosa*. It causes wound and urinary tract infections and is an important cause of nosocomial infections.	Pseudomonas infections are common in burn and immunosuppressed patients. *P. aeruginosa* grows at room temperature and produces a water-soluble blue-green pigment. *P. aerugenosa* is the prototype of a group of organisms termed nonfermenters since they are generally aerobic and fail to ferment glucose.	*P. aeruginosa* may be grown on Trypticase soy agar to show blue-green pigment. The strictly aerobic nature of its growth may be observed in broth or semisolid media in a tube where it only grows near the surface.
15.532 *Francisella (Pasteurella) tularensis*	This organism produces tularemia or rabbit fever, a disease of rodents transmitted by various arthropods such as certain flies, ticks, and lice. Humans develop the infection after handling, eating, or skinning infected animals or by drinking water contaminated by them. The bacilli enter the host via the arthropod bite or through the respiratory or gastrointestinal tract. The disease is produced by rapid invasion of the host and multiplication of the bacilli in many tissues.	Clinical disease may be localized at the portal of entry such as the eye, skin, or lungs where granulomatous lesions occur. Bacteremia with pneumonia and septicemia may follow. Diagnosis is by serology or, in acute cases, repeated blood cultures may allow cultivation of the organism. Tularemia can be prevented by taking proper precautions with wild animals and by adequately cooking infected meat. Control measures include control of rodent populations and surveillance of animals.	
15.533 *Brucella*	The brucellae are obligate parasites of animals which cause undulant fever in humans. The infectious agents are primarily the animal pathogens *B. abortus* in cows, *B. suis* in pigs, *B. melitensis* in goats, and *B. canis* in dogs. In animals the bacilli are localized in the mammary glands and transmitted via milk, or they are localized in the genitalia. Farmworkers or veterinarians may become infected by handling contaminated material. Sensitivity to endotoxin may play a role in the pathogenesis of brucellosis.	The disease in humans is characterized by a long-continued bacteremia with a remittent "undulant" fever. Organisms multiply inside the cells of the reticuloendothelial system, allowing persistence of the organism in spite of high-level chemotherapy. Diagnosis is by repeated cultivation using special media and techniques for this slowly growing organism or by serology. Prevention is by slaughter of infected animals and immunization of animals with a live vaccine plus compulsory pasteurization of milk and milk products.	

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			Long-term treatment with tetracyclines or ampicillin may be effective.	
15.534	*Bordetella*	*B. pertussis* is the etiological agent of whooping cough. Virulent strains are encapsulated. *B. pertussis* is a fastidious organism requiring special media for primary isolation. On Bordet-Gengou agar the colonies are raised and have a characteristic sheen, like a mercury drop. The bacilli possess endotoxin which is probably responsible for degenerative changes in the respiratory tract. Prevention is with a vaccine of killed organisms given especially to infants and young children (DPT).	Whooping cough consists of a catarrhal stage with release of a large number of organisms and a paroxysmal stage with an explosive whoop on inhalation. Laboratory diagnosis is by cultivation of nasopharyngeal exudate and immunofluorescent staining. Treatment includes early use of erythromycin and ampicillin.	
15.54	Gram-Negative Anaerobic Rods (*Bergey's #9*)	Anaerobic, gram-negative, non-sporulating bacilli represent the most common anaerobes encountered in infections.	Elaborate procedures are required for recovery of these organisms from clinical specimens as well as identification.	Projection slides and films of methods of cultivation may be studied.
15.541	*Bacteroides*	*B. fragilis* is the most common anaerobe strain isolated. It represents 95% of the bacteria of the intestinal tract and is responsible for the foul odor of feces. In anaerobic infections, bacteroides are usually found in association with other organisms.	*B. fragilis* causes abscesses, appendicitis, bacteremia, and heart valve infections. Most strains are sensitive to erythromycin and clindamycin but resistant to penicillin.	Anaerobe jars or pictures of transport anaerobes may be demonstrated.
15.55	Gram-Negative Rods (Unclassified)	The Legionnaires bacillus was isolated and identified after the publication of *Bergey's Manual* and so is not placed in one of the other categories.		
15.551	*Legionella pneumophila*	These Gram-negative, pleomorphic bacilli are responsible for a very severe form of pneumonia called Legionnaires disease. The organisms can live saprophytically in stagnant water.	*Legionella* requires enriched media and is oxidase and catalase positive. Different antigenic strains have been found. It can be definitely identified only by the direct fluorescent antibody test. Treatment is with erythromycin or tetracycline.	
15.6	The Spirochetes (*Bergey's #5)*	Spirochetes are thin-walled, flexible, motile, helical rods. These microorganisms may be seen readily in the darkfield microscope.	The spirochetes are actively motile, rotating around their long axes.	Material scraped from around the teeth will reveal nonpathogenic spirochetes. Electron micrographs showing the special structures of the spirochetes may be shown.

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15.61	*Treponema*	*T. pallidum* causes syphilis, which is transmitted by sexual or direct contact. This pathogenic organism has never been cultured. Syphilis is a disease of varying duration. The primary "hard chancre" at the site of the infection heals and may be followed by a secondary stage characterized by a rash. The skin lesions are highly contagious and mimic a variety of other dermatological diseases. A later or tertiary stage may involve granulomatous lesions of any part of the body or cause degenerative changes in the central nervous system or cardiovascular system.	A pregnant syphilitic woman can tranamit the spirochete to the fetus through the placenta. This may result in death of the fetus or a variety of birth defects. Early adequate treatment prevents congenital syphilis. Laboratory diagnosis includes serology using non-treponema antigen. Control of the disease includes treatment of all cases, follow-up of all contacts, and education. Serological tests for syphilis are usually required to obtain a marriage license, on all hospital admissions, and during early pregnancy. *T pallidum* is extremely susceptible to penicillin.	Electron micrographs of the organism and projection slides demonstrating lesions may be shown.
15.62	*Leptospira*	*L. interrogans* is a tightly coiled spiral microorganism having one or both ends bent into a hook. These organisms can be cultured on media containing serum. The leptospira are primarily pathogens of the urinary tract of animals such as dogs, rats, and cattle which excrete the organisms in their urine. Human infection is acquired by ingestion of food or water containing the organisms or less commonly by direct penetration of the skin. Many infections are mild or subclinical.	Leptospirosis (Weil's disease) produces hemorrhage and necrosis in the liver and kidneys and results in dysfunction of these organs. Diagnosis includes darkfield examination, culture, and serology. Prevention includes avoiding ingestion of contaminated water, control of rodents, and vaccination of dogs in endemic areas. Penicillin and tetracyclines are helpful but do not eradicate the infection.	
15.63	*Borrelia*	*B. recurrentis* is the etiological agent of relapsing fever, a tick- and louse-borne disease. The organism is an irregular spiral that stains readily with bacterial stains. The disease is characterized by 3 to 10 bouts of sudden fever followed by chills and weakness. The disease is endemic in areas in which the particular tick carriers are found.	Rodents are the main reservoir for the infection. They transmit the spirochete to ticks that may transmit them to humans or transovarially to other ticks. The human louse may transmit the disease from human to human. Prevention is by avoidance of exposure to ticks. Blood smears obtained during the fever may reveal the organisms.	
15.71	*Campylobacter fetus*	*Campylobacter fetus* subspecies *jejeuni* is a motile gram-negative curved rod which is a major cause of acute gastroenteritis. It causes a rapid-onset diarrheal disease which	Stool specimens must be inoculated on a highly selective media, incubated under reduced oxygen tension and incubated at 42°C in order to isolate campylobacter. They are	

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	is self-limited and lasts less than a week. These organisms are strict microaerophiles and grow at 42°C.	generally susceptible to tetracyclines, erythromycin, and aminoglycosides.	
15.8 The Mycoplasmas (*Bergey's* #19)	The mycoplasmas are small highly pleomorphic microorganisms which do not have a cell wall. They require special staining methods to be seen. Mycoplasmas have an affinity for cell membranes, are found in plants and animals, and are part of the normal flora of the mouth and genitourinary tracts of humans. The mycoplasmas are highly host specific. *Mycoplasma pneumoniae* is the only species proven to be a human pathogen and causes atypical pneumonia. *Ureaplasma urealyticum* (T strain of mycoplasma) hydrolyzes urea and is thought by some to be associated with nongonococcal urethritis.	Mycoplasmas do not revert to walled type forms. These fastidious organisms can reproduce on cell-free media. On agar, colonies of certain species are characteristically beneath the surface giving a "fried egg" appearance. Penicillin and thallium acetate may be added to media used in the primary isolation of mycoplasmas to inhibit growth of other bacteria. Serological tests are available. Tetracyclines and erythromycin are used for treatment.	
15.9 The Rickettsiae (*Bergey's* #18)	The rickettsiae include the orders *Rickettsiales* and *Chlamydiales*.		
15.91 *Rickettsiales*	The *Rickettsiales* are extremely small gram-negative obligate, intracellular parasites of arthropods. They are transmissible to humans by the arthropods, with the exception of Q fever, which does not require an arthropod vector. Rickettsial infections are generally characterized by fever and rash.	The *Rickettsiales* stain poorly with Gram stain but can be seen with Giemsa stain. Rickettsia may be cultivated on the yolk sac of embryonated eggs, in tissue cell culture, or in experimental animals. Diagnosis is by specific serological tests or by the Weil-Felix test in which antibodies produced against rickettsias cross react with *Proteus* sp. Treatment is with tetracyclines and chloramphenicol.	Prepared microscope slides of rickettsiae in chick embryo tissue smears may be studied. Projection slides of the vectors and patients with the disease may be shown.
15.911 *Rickettsia*	*R rickettsii* is the etiological agent of Rocky Mountain spotted fever. The bacteria are transmitted from tick to tick (transovarially) or from tick to humans. The rickettsiae grow in endothelial cells of small blood vessels causing obstruction, thrombosis, and necrosis of the skin (pro-	Rocky Mountain spotted fever occurs in deer, rabbits, and other vertebrates which live in heavily wooded areas where the ticks occur. Most Rocky Mountain spotted fever now occurs in the eastern and southeastern states primarily in the late summer or fall. Prevention is by avoidance or control of	

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	ducing a rash), the heart, and central nervous system.	ticks by clearing heavily wooded areas. A vaccine for the disease is available. Epidemic typhus is a disease restricted to humans. It is caused by *R. prowazekii* and transmitted by the human louse. It is not found in the United States at the present time. Endemic typhus, caused by *R. typhi*, is transmitted by the rat flea from the rat to human. It is prevalent worldwide, especially in seaports. Q fever caused by *Coxiella burnetti* is a disease resembling influenza, atypical pneumonia, and hepatitis. Transmission is via the air instead of through the skin. It is found worldwide.	
15.92 *Chlamydiales*	These organisms are small gram-negative obligately intracellular bacteria which multiply in the cytoplasm of the host cell. Although they are gram negative, they are visualized better by Giemsa or other stains. They undergo a developmental cycle from the infectious particle to the larger initial body.	Subclinical infections are the rule as the parasite reaches a balance with the host, while spread of the infection from one species to another is more apt to produce disease. Diagnosis is by serological tests or cultivation of the organism in embryonated eggs or tissue culture cells. Treatment is with tetracyclines.	
15.921 *Chlamydia*	The two species of cylamydia are *C. psittaci* and *C. trachomatis*. *C. psittaci* causes ornithosis or psittacosis in humans. The disease is acquired by inhalation of dried bird excreta or handling contaminated plumage. *C. trachomatis* causes lymphogranuloma venereum, a venereal disease reported most commonly from the tropics and subtropics but found worldwide. Trachoma, the world's leading cause of blindness and inclusion conjunctivitis are also caused by *C. trachomatis*. About 50% of the cases of nongonococcal urethritis are caused by *C. trachomatis*.	Psittacosis in humans varies from a severe, even fatal pneumonia to a mild inapparent infection. Birds imported from South America and the Far East are quarantined to prevent entry of this disease. The inguinal lymph nodes in lymphogranuloma venereum may become inflamed, suppurate, and discharge pus, especially in males. Trachoma is spread from eye to eye by fingers, fomites, and flies in areas of the world where poor sanitary conditions prevail. Nongonococcal urethritis may coexist with other venereal diseases.	

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16.0 Medical Mycology			
16.1 General Consideration	Most pathogenic fungi are inhabitants of the soil. Invasion of the host is by direct contact, resulting in skin lesions, or by inhalation of spores, resulting in respiratory or systemic infections. *Candida albicans* is the only pathogenic fungus which is part of the normal flora. It is not an inhabitant of the soil. Diseases caused by fungi are called mycoses.	Of the thousands of species that exist, fewer than 100 are considered pathogenic.	Prepared projection slides of cultures or developmental stages of the pathogenic fungi or lesions may be studied. *Aspergillus*, the *Phycomycetes*, *Saccharomyces*, and various other fungi found in the environment may be safely cultured in the student laboratory. Cultivation of pathogenic fungi should not be attempted.
16.12 Diagnosis	Diagnosis of the mycoses is made by means of one or more of the following methods: isolation of the etiological agent in culture media with subsequent identification; microscopic examination of exudates or skin scrapings; microscopic examination of histopathological slides; examination of the skin or hair for fluorescence; and serological techniques.	Cultivation of pathogenic fungi is very hazardous and should be performed only by well-trained specialists using appropriate safety cabinets.	
16.13 Pathogenesis	Pathogenic fungi generally do not produce toxins but induce hypersensitivity in the host. In the mycoses of deep, or internal, tissue, the tissue reaction commonly is a chronic granuloma. Human mycotic infections are more conveniently grouped by type of infection (superficial, subcutaneous, and systemic) rather than by organism.	The diseases produced in deep tissues resemble those produced in chronic bacterial infections, especially those produced by the mycobacteria, e.g., tuberculosis.	
16.2 Superficial Mycoses	These fungi invade only hair and the outer layer of the epidermis. Since the host does not produce a response such as redness, itching, or swelling, the problem is often only cosmetic.	Causal organisms and disease are *Cladosporium werneckii*, which causes tinea nigra (palmaris), *Piedraia hortai*, which causes black piedra, *Trichosporon cutaneum*, which causes white piedra, and *Malassezia furfur* which causes tinea versicolor.	
16.3 Cutaneous Mycoses (Dermatophytes)	The dermatophytes are fungi which infect skin, hair, or nails by means of direct hyphal penetration. They are not dimorphic. In skin lesions, these fungi generally look alike, showing hyphae and arthrospores. Some species are found primarily in human skin, some in domestic and wild animals, and a few are found as saprophytes in the soil.	A cutaneous mycosis is described clinically by the area of the body affected; i.e., tinea corporis is ringworm infection of the body, tinea pedis is athlete's foot, and tinea capitis is ringworm infection of the scalp. Therapy often consists of topical application of antifungal agents over an extended period of time. In widespread involvement	

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	Human to human and animal to human transmission is common for some diseases. The dermatophytes produce chronic, low-grade infections. Skin lesions show inflammation at the site of the actively growing hyphae found on the periphery of the lesions. This gives the typical ring-type lesion of "ringworm."	of hair or nail infection griseofulvin is the drug of choice. *Microsporum* may be found on skin and hair, *Epidermophyton* may be found on skin and nails and *Trichophyton* infects skin, hair, and nails. Most species are found worldwide, but a few are localized. There is some age variation as to the type of disease produced, i.e., athlete's foot is more common in adults, while scalp infections are more common in children.	
16.4 Subcutaneous Mycoses	The subcutaneous mycoses are caused by certain organisms introduced via a break in the skin (splinters, thorns, etc.). Lesions involve skin, subcutaneous tissue, fascia, and bone. Once established, these fungi tend to remain localized.		
16.41 Sporotrichosis	Sporotrochosis is caused by *Sporothrix schenckii*, which is a dimorphic fungus found worldwide on plants or plant structures. In sporotrichosis, a local ulcerated lesion develops at the site of inoculation and may progress to the formation of multiple nodules and abscesses along the superficial draining lymphatics, usually along the arm or leg.	In infected tissue, the fungus appears as a cigar-shaped, budding yeast. The disease may rarely progress from subcutaneous lesions to the respiratory tract or by extension to bones and joints. Sporotrichosis, while not a common disease, occurs mostly in gardeners, florists, and others having contact with plant materials. Treatment is oral potassium iodine for the subcutaneous lesions or amphotericin B for the systemic disease.	
16.42 Chromomycosis	Chromomycosis is caused by a group of slowly growing, dimorphic fungi. Chromomycosis is an infection characterized by slowly developing cauliflower-like lesions along the lymphatics of the feet or legs and is caused by several different black molds.	In lesions, the fungi appear as thick-walled brown cells which multiply by fission. On cultivation the fungi appear as darkly pigmented, slowly growing mycelial colonies. Fungi isolated include: *Phialophora verrucosa, Fonsecaea pedrosoi*, and *Cladosporium carrionii*.	
16.43 Mycetoma	Mycetoma is a fungus nodule or tumor which may be caused by filamentous fungi (Eumycetoma or Maduramycosis) or certain branching bacteria (Actinomycetoma). The disease consists of a localized lesion, often on the foot, with draining sinus tracts which	A mycetoma originates from an injury with resulting inoculation of microorganisms into the body. Identification as to fungal or bacterial agent is important because of the differences in treatment. Treatment includes sulfonamides and penicillins for the	

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	yield granules consisting of hyphae or fragments of bacterial filaments.	actinomycotic mycetoma. There is no established treatment except surgical removal for the fungal mycetoma.	
16.5 Systemic Mycoses	The systemic mycoses are diseases of the internal tissues usually caused by dimorphic fungi which grow as soil saprophytes. Infection is primarily acquired by spore inhalation, with most infections being asymptomatic. The chronic form starts with a pulmonary lesion and spreads via the bloodstream or by direct extension. A small percentage of cases are fatal. There is no human to human or animal to human transmission.	In addition to the usual methods of diagnosis of mycoses, histopathology is especially important in systemic infections. Treatment of all these diseases is with prolonged administration of amphotericin B. A variety of domestic and wild animals may be found infected with the systemic mycoses.	
16.51 Coccidioidomycosis	*Coccidioides immitis* is present in tissue as thick-walled spherules containing up to several hundred endospores. Spherules rupture, releasing endospores which start their own spherules. The major endemic area in the world is the southwestern United States where *C. immitis* grows abundantly in desert areas.	In culture, the fungus is characterized by arthrospores which alternate with clear hyphae. Infection is through inhalation of arthrospores. The resulting respiratory infection may be asymptomatic or influenza-like or 1% may progress to the disseminated, highly fatal form. Very high percentages of all of the population of the endemic areas have positive skin tests showing previous infections.	
16.52 Histoplasmosis	*Histoplasma capsulatum* grows in soil, especially those enriched with blackbird or chicken droppings. In tissue it is seen as small yeasts in macrophages and cells of the reticuloendothelial system. The endemic area is predominately along the Mississippi and Ohio River Valleys where 90 to 95% of the people show positive skin tests. The infectious form produces microconidia which enter the body through the lungs. In vivo these spores change into yeast cells.	Histoplasmosis is a disease of the lungs and reticuloendothelial system. Initial infection is often mild and self-limiting or may produce granulomatous lesions and a disease which may resemble tuberculosis and is a major cause of lung calcification. Supportive therapy usually is sufficient for primary pulmonary histoplasmosis. Systemic histoplasmosis is treated with amphotericin B. A reliable skin test is available.	
16.53 Blastomycosis	*Blastomyces dermatitidis* appears in tissue or pus as a thick-walled nonencapsulated yeast with broad-based buds. Blastomycosis is usually restricted to the upper Mississippi and Ohio River valleys where it is thought	Blastomycosis, a disease of skin, lungs, and bone, may be asymptomatic or develop into a serious pulmonary disease resembling tuberculosis or cancer. Paracoccidioidomycosis, a somewhat similar disease, caused by	

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	to be a soil saprophyte. The infectious phase probably produces spores which enter the body by the lungs. In vivo these spores change into yeast cells.	*Paracoccidioides braziliensis* is not found naturally in the United States.	
16.54 Cryptococcosis	*Cryptococcus neoformans* is the only pathogenic fungus found as an encapsulated yeast in tissues. This organism grows on soil, especially in the presence of pigeon excrement. Pulmonary disease is the most common form and may be asymptomatic. In more severe cases, the organism has a predilection for the brain and meninges. There is little or no inflammatory reaction but sometimes granulomatous lesions are found. The capsule of this yeast inhibits phagocytosis.	Cryptococcosis is found worldwide, being more prevalent in urban areas with pigeon populations. Treatment is with 5-fluorocytosine and/or amphotericin B. Diagnosis of cryptococcal meningitis involves detection of encapsulated yeast in the cerebrospinal fluid by microscopic examination with India ink and/or cryptococcal antigen detection.	
16.6 Opportunistic Mycoses	Opportunistic mycoses are caused by fungi which are part of the normal flora of the individual or are present in the environment but do not produce disease except in special circumstances, such as a debilitated state of the host or an overwhelming exposure to spores by healthy individuals.	Severe diabetes, malignant tumors of the lymphoid tissue, extensive treatment with broad-spectrum antimicrobial agents or with agents which suppress the immune response (corticosteroids, X-irradiation, cytotoxic drugs) all predispose an individual to the opportunistic mycoses.	
16.61 Candidiasis (Candidosis)	*Candida albicans* is a budding yeast which may produce hyphae or pseudohyphae in tissues. It is found as part of the normal flora of the mucous membranes of the mouth, vagina, and intestinal tract in a high percentage of the population. Disease may occur in many parts of the body and from a variety of causes of lowered resistance. Diseases include thrush, vulvovaginitis, infections around nails, or systemic infections in immunosuppressed individuals.	Disease may be produced on the mucous membranes or the skin. *Candida* may be transmitted between individuals, from mother to baby as the baby passes through the birth canal, or by fomites. Other species of *Candida* may be present as members of the normal flora and more rarely cause disease. Candidiasis is a common cause of death in cancer and transplant patients. Treatment is with nystatin locally or amphotericin B for systemic infections.	
16.62 Aspergillosis	A large number of species of the aspergilli which are distributed widely in nature and include many plant pathogens produce opportunistic infections. Aspergillosis is usually a disease of the respiratory tract and results in either suppurative, or granuloma-	Patients with lung lesions from a previous tuberculosis infection, farmers and others handling dead and decaying vegetation are most apt to acquire aspergillosis. Certain *Aspergillus* species prevalent on stored grain or peanuts produce mycotoxins, which	

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	tous lesions. In tissue, *Aspergillus* species produce septate hyphae with dichotomous branching. *A. fumigatus* is the most common pathogenic species.	are highly toxic when ingested and may be carcinogenic. Since the aspergilli are found commonly as contaminants on laboratory media, repeated isolations and identifications may be necessary to establish them as etiological agents of disease in humans.	
16.63 Zygomycosis (Mucormycosis, Phycomycosis)	*Mucor* or *Rhizopus* are occasionally found in tissues of diabetics and immunologically compromised individuals. These organisms aggressively grow toward, and may produce occlusions in blood vessels.	The lesion may be one of acute inflammation or show little tissue response. Diagnosis is made by finding the large non-septate hyphae in tissue or by repeated cultivations. Metabolic acidosis is a predisposing factor for acute zygomycosis.	
17.0 Medical Virology			
17.1 Epidemiology			
17.11 Portals of Entry	The primary routes of viral entry in the human host include the skin, the respiratory and gastrointestinal tract mucosa, and, to a lesser extent, the eye and genitourinary tract. Certain viruses may be transmitted in utero.	Examples of specific viruses and their routes of entry include: warts—skin; influenza—respiratory mucosa; polioviruses—gastrointestinal mucosa; adenoviruses—mucous membranes of the eye; herpes simplex type 2—genitourinary tract; and rubella—placenta.	
17.12 Transmission	Inapparent infections are the most important source of virus transmission from human to human. Viruses may be transmitted by healthy individuals harboring latent viruses.	Humans are the only host for many viruses such as mumps, measles, and polio, whereas other viruses such as rabies are transmitted to humans from other animals.	
17.13 Periodicity	Many viral diseases such as the common cold are seasonal whereas others such as hepatitis B, herpes simplex, and rabies are not. Some seasonal viruses such as the rhinoviruses cause epidemics yearly, whereas measles and influenza A virus tend to cause epidemics at 2- to 3-year intervals.	Many viruses remain endemic and others remain sporadic in a given population.	
17.2 Disease States	Most pathogenic viruses cause acute infections. Certain viruses however, may enter a host and remain latent indefinitely or may become clinically expressed at intervals later	Measles is an example of an acute viral disease. Fever blisters are caused by a virus (herpes) which remains latent and is expressed clinically at infrequent intervals.	

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	in life. Slow viruses may enter a host and incubate for months or years before symptoms appear.	Subacute sclerosing panencephalitis (SSPE) is an example of a slow virus infection.	
17.21 Localized Infections	Many respiratory viruses invade the mucous membrane of the respiratory tract and replicate and produce their clinical manifestations at this primary site.	Examples are the rhinoviruses and other cold-causing viruses.	
17.22 Systemic Infections	Many viruses invade the mucous membranes of the respiratory or gastrointestinal tracts and, after replication, enter the lymphatic system where they continue to replicate. The virus is then released into the blood resulting in a viremia. The circulatory system delivers the virus to its target organ with resultant clinical disease.	Examples of such viruses include poliovirus, measles virus, and chickenpox virus.	
17.3 Response of the Host to Viral Infection	Host response generally includes inflammation involving phagocytic cells, interferon production, and a specific immune response. Mononuclear cells (macrophages) are the primary phagocytic cells involved, whereas bacterial infections elicit a polymorphonuclear cellular response. Interferon is a protein produced by a virus-infected cell and indirectly interferes with continued virus replication in other cells. It is important in recovery from viral disease. The cell-mediated immune response (T lymphocytes) appears to be primarily responsible for host recovery from viral infection, whereas the humoral immune response (B lymphocytes) seems to be primarily involved in the prevention of reinfection.	Interferon induces cells to form translation inhibitory protein (TIP) which prevents the translation of viral mRNA. Interferon is tissue and host specific but is not specific for a particular virus.	
17.4 Antiviral Agents	Viruses have no inherent metabolic capability but rely on host cellular metabolism for replication. Antiviral agents are therefore generally directed toward inhibition of viral nucleic acid synthesis and/or function.	Idoxuridine (IDU) and adenine arabinoside (ara-A) are nucleic acid antagonists useful against certain herpes simplex infections. Amantadine is indicated for the prevention and/or treatment of influenza A and the thiosemicarbazones have been successfully used against smallpox.	

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17.5 Immunoprophylaxis	Live vaccines are superior to killed vaccines in promoting high levels of lasting immunity. Killed vaccines require booster doses to maintain adequate immunity, whereas single injections of live attenuated vaccines provide a lasting immunity. A variety of viral vaccines, both killed and live-attenuated, are commercially available.	Killed vaccines are available for influenza and rabies. Live attenuated vaccines are available for polio, rubeola (measles), and rubella (German measles).	
17.6 Diagnostic Virology	Viral diseases may be diagnosed in the laboratory either by virus isolation or by detecting a rise in specific antibody.		
17.61 Virus Isolation	The presence of virus in cell culture systems can be detected by separation and rounding of cells from a monolayer (cytopathogenic effect, or CPE), the interference of cytopathogenesis, the ability of certain virus-infected cells to adsorb erythrocytes onto their surface (hemadsorption) or the ability of virus-containing fluids from cell culture systems to hemagglutinate erythrocytes.		Projection slides of CPE in cell culture systems and slides of positive hemadsorption in cell culture systems may be studied.
17.62 Virus Identification	Virus isolated in cell culture systems can be identified by a variety of tests utilizing specific known antisera to the suspected virus, which will inhibit the expression of the virus in the cell culture system.	If measles antibody is mixed with fluids suspected of containing measles virus and this mixture is then inoculated into a cell culture system the CPE, which would characteristically be caused by the measles virus, will be prevented from occurring. Similarly, if influenza antibody is mixed with influenza virus and inoculated into a susceptible cell culture system, the virus will be incapable of growing and the adsorption of erythrocytes to the cell surface will be prevented.	
17.63 Serological Diagnosis	To diagnose a viral disease serologically, a significant elevation in specific antibody level between an acute and convalescent serum should be demonstrated. The acute serum should be collected immediately after the onset of the disease, and the convalescent serum, with some exceptions, should be collected 10 to 14 days later.	Techniques for the detection of specific antibody levels in a patient's serum include hemagglutination inhibition, complement fixation, immunofluorescence antibody, and radioimmunoassay.	

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17.7	RNA Viruses			
17.71	Picornaviruses	Picornarviruses are small (pico) RNA viruses.		
17.711	Enteroviruses	Enteroviruses initially replicate in the gastrointestinal tract and are excreted in the stool.	Enteroviruses when swallowed can find their way to the gastrointestinal tract since they are acid stable and are not destroyed by the acidity of the stomach.	Projection slides of an enterovirus rash may be shown.
17.7111	Polioviruses	The three antigenically distinct polioviruses may spread from the gastrointestinal tract via blood and infect the spinal cord and/or brain, which results in limb and/or respiratory paralysis. Polioviruses may cause viral meningitis and upper respiratory tract infections but most commonly cause subclinical infections. The Sabin trivalent live oral polio vaccine has virtually eliminated paralytic disease in immunized populations. The Salk vaccine uses inactivated poliovirus.	Viral meningitis involves inflammation of the meninges (the coverings of the brain and spinal cord). It is usually a self-limiting disease. Other enteroviruses include the echoviruses and coxsackie A and B viruses. These latter three groups may be associated with a raised (papular) rash.	
17.712	Rhinoviruses	There are over a hundred antigenically distinct rhinoviruses, all of which may cause the common cold.	In contrast to other human viruses, rhinoviruses propagate best at 33°C rather than 36° to 37°C.	
17.72	Togaviruses (Arboviruses)	Togaviruses are arthropod (vector) transmitted and are comprised of two serologically distinct groups, the alphavirus group and flavivirus group.	The equine encephalitides, yellow fever, and dengue are some of the important viruses in this group. They are mosquito transmitted.	
17.721	Rubella (German Measles)	Rubella is a mild rash-associated disease which belongs to the togavirus group but is not vector transmitted. If the virus is contracted during the first 3 months of pregnancy, infection of the fetus may result in death or birth defects such as cataracts and heart abnormalities. The use of the live rubella vaccine has significantly decreased the incidence of rubella-caused birth defects.	Women of childbearing age should be checked for antibodies to rubella. If they have none they should be immunized. Pregnant women should not be immunized with the rubella vaccine.	
17.73	Orthomyxoviruses			

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17.731 Influenza A	Influenza A is the most common cause of influenza in humans, occurs in epidemic form every 2 to 3 years, and is characterized by sudden onset of headache, fever, achiness, and minimal respiratory symptoms.	The antigenicity of the influenza A virus changes periodically. For instance, the Hong Kong strain may be present for a time and then another antigenic strain, such as the Russian strain, may take over. Influenza B and C are similar clinically to influenza A but epidemiologically are not as significant.	
17.74 Paramyxoviruses			
17.741 Mumps	The parotid glands are the target organs of the mumps virus. Occasional complications of the young adolescent include inflammation of testes and the heart muscle. The live mumps vaccine given early in life has significantly decreased the incidence of complications.	Mumps is the most common cause of postinfectious encephalitis; however, the encephalitis is usually of little clinical significance. Postinfectious encephalitis is an immune type of disease, as opposed to a primary encephalitis which results in virus invasion of nervous tissue.	Projection slides of a case of mumps may be shown.
17.742 Parainfluenza Viruses	There are four antigenically distinct parainfluenza viruses. They are responsible for croup, an asthmatic type of bronchitis (bronchiolitis), pneumonia, and upper respiratory tract infections in infants.		
17.743 Measles (Rubeola)	Measles is a common disease of childhood and is associated with raised lesions (a papular rash). Measles is associated with a postinfectious encephalitis which may result in permanent brain damage or death in the very young. The live measles vaccine given during infancy has significantly decreased the incidence of encephalitis.	Other complications of measles include middle ear infection, bronchitis, and pneumonia. Combination live virus vaccines (measles-rubella, rubella-mumps) are available.	
17.744 Respiratory Syncytial Virus (RSV)	RSV causes pneumonia and bronchiolitis in the very young. It may cause epidemics, usually in the winter.		
17.75 Rabies	Rabies, a rhabdovirus, is transmitted to humans by the bite of a rabid animal. The virus contained in the saliva reaches the central nervous system via the traumatized nerves. The disease is characterized by a long incubation period and is almost 100% fatal. Rabies therapy involves the use of	A new nonallergenic vaccine prepared in human fibroblast cells (cell cultures) and requiring fewer injections provides a longer-lasting immunity.	

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	antirabies serum and the daily injection of killed duck embryo vaccine (DEV) for 14 to 21 days. In the United States rabies is most commonly found in skunks and, to a lesser degree, in raccoons and other wild animals, as well as in a few domestic animals and bats.		
17.8 DNA Viruses			
17.81 Papovaviruses	The human papilloma virus (wart virus) causes the characteristic juvenile and/or plantar warts. Venereal warts are sexually transmitted and may undergo cancerous change if untreated.	Viruses from maternal genital warts may be acquired at the time of birth by infants who may later develop tumorous growth of the vocal cords. Treatment of warts is by surgical or chemical removal or by cauterization.	
17.82 Adenoviruses	The 31 antigenically distinct adenoviruses cause respiratory illness and/or conjunctivitis.	A live oral vaccine incorporating types 4 and 7 is used in military recruits where infection rates are higher than in the lay population. Some human adenoviruses cause cancer when injected into laboratory animals.	
17.83 Herpes Group of Viruses	This group includes viruses which, following primary infection, may remain in the host for life.		
17.831 Herpes Simplex Virus Types 1 and 2 (HSV-1 and HSV-2)	HSV-1 is the cause of the common fever blister or cold sore. It may also cause infections of the eye and brain. HSV-2 is sexually transmitted and causes genital lesions. Infection of the fetus or newborn may be fatal.	HSV-1 infections occur early in life and by adulthood 70 to 90% have type 1 antibodies. HSV-2 infection usually occurs after puberty or is acquired during birth.	Projection slides showing HSV-1 and HSV-2 infection may be shown.
17.832 Varicella-Zoster Virus (V-Z Virus)	The V-Z virus causes chickenpox in children and shingles in adults. The chickenpox virus remains latent in the host and may be expressed as shingles in later life following some predisposing factor.	Shingles in the adult can be the source of chickenpox in children and initiate large outbreaks. Shingles may be associated with spinal cord trauma or malignancy.	Projection slides of cases of chickenpox and of shingles may be shown.
17.833 Epstein-Barr Virus (EB Virus)	The EB virus is thought to be the etiological agent of infectious mononucleosis and is possibly associated with Hodgkin's disease.	The EB virus was first associated with Burkitt's lymphoma, a malignant tumor of the jaw which occurs in African children and	

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			nasopharyngeal carcinoma common in Chinese males.	
17.834	Cytomegalovirus (CMV)	CMV is endemic in humans and causes subclinical infection in otherwise healthy individuals. CMV may cause congenital abnormalities of newborns, post-transfusion mononucleosis and pneumonia, eye infections, and other systemic complications in patients undergoing organ transplant or on immunosuppressive medication.	Antibody to CMV is found in 80% of individuals over 35 years of age. The virus may be excreted intermittently by a host with latent infection.	Projection slides of congenital CMV infections may be shown.
17.84	Poxviruses	The poxviruses are large, have a complex symmetry, and include agents affecting a variety of animals, including humans.		
17.841	Variola (Smallpox)	Smallpox is characterized by a vesicular eruption of the skin and is associated with a high morbidity and mortality. Through the efforts of the World Health Organization the world today is essentially smallpox free. Smallpox immunization is required only for those returning to the United States from certain risk areas.	In 1798, Jenner was the first person to use immunization procedures to prevent disease. He used cowpox to protect against smallpox. A laboratory strain of cowpox (vaccinia) is now used as the agent in smallpox immunization.	Projector slides showing classical smallpox and the various stages of smallpox immunization may be shown.
17.9	Other Less-Well-Defined Viruses			
17.91	Hepatitis A Virus (HAV)	HAV is ubiquitous in that most adults have had exposure to it. It is transmitted primarily from person to person by the fecal-oral route, although epidemics have been associated with infected shellfish and contaminated drinking water. Clinical manifestations involve the liver although the disease is usually of a mild nature.	About 30 to 60% of American adults have antibody to HAV and an even higher prevalence occurs in lower socioeconomic groups.	
17.92	Hepatitis B Virus (HBV)	HBV is transmitted by blood and blood by-products and can also be transmitted by other routes. Contaminated needles of drug addicts are also a common method of transmission. HBV clinically is similar to HAV although it tends to be a more serious disease. A small percentage of infected individ-	All donated blood is screened for the HBV surface antigen. Most of the hepatitis that results from blood transfusions is not due to HAV or HBV but to a nonA-nonB virus and perhaps yet unknown viruses.	

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		uals may become chronic carriers and remain potentially infectious to others.		
17.93	Slow Viruses Infecting Humans	Kuru, Creutzfeldt-Jakob disease, subacute sclerosing panencephalitis (SSPE), and progressive multifocal leukoencephalopathy (PML) are examples of diseases caused by slow viruses. They produce a neurological disease which is progressively fatal. These diseases are characterized by long incubation periods (months to years).	Kuru, a disease of the New Guinea highlands, is transmitted by ingestion of undercooked infected human brain and is on the decline due to a decrease in cannibalism.	
18.0	**Medical Parasitology**			
18.1	General Features of Protozoan Infections			
18.11	Transmission of Protozoa	The life cycle of protozoa found in the mouth and intestinal and urogenital tracts is usually simple and direct, i.e., the protozoa are in the infective stage shortly after they leave the host. Protozoa which invade blood and other tissues generally have an indirect life cycle in that certain developments of the organisms take place in intermediate hosts such as insects before the protozoan is infectious for humans.		Diagrams and charts depicting the life cycles and transmission characteristics of medically important protozoa may be studied.
18.12	Pathogenesis of Protozoan Diseases	Protoza affect the host by multiplication of the organisms, invasion of tissue, and production of toxins. In certain cases, the host develops a hypersensitivity to the foreign protozoan protein, resulting in an inflammatory reaction. Intestinal parasites produce less local and systemic effects than tissue parasites.	Many pathogenic protozoa secrete proteolytic enzymes, hemolysins, cytolysins, and other toxins which aid in their virulence.	
18.13	Symptoms of Protozoan Infection	Protozoan infections may be subclinical, chronic, or acute. Often, an acute disease may result in a chronic infection.	More than 90% of all protozoan infections are asymptomatic, showing mutual tolerance between the host and parasite.	
18.2	Medical Protozoology			
18.21	Sarcodina, the Amoebae	Most intestinal amoebae are nonpatho-	*Entamoeba coli* and *Endolimax nana* are	

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	genic and occur as commensals in the cecum and colon. They must be differentiated from the only primary pathogenic species *Entamoeba histolytica.*	commensals in the large intestine of humans and are only rarely implicated in disease. Their presence indicates that an individual has ingested something contaminated with feces.	
18.211 *E. histolytica*	*E. histolytica* is the most important pathogenic amoeba which infects humans. Infection is acquired by ingestion of cysts from contaminated food or water. Infection often results in asymptomatic carriers, but in disease caused by invasion of colon wall, resultant tissue destruction occurs accompanied by diarrhea or dysentery. Extraintestinal disease, while rare, may occur in the liver or other organs.	Important sources of infection are asymptomatic carriers. Fresh vegetables fertilized with human feces are sources in certain foreign countries. Trophozoites may be seen in runny stools while cysts are more common in formed stools. Extraintestinal disease may be detected serologically. Metronidazole is generally the treatment of choice for extraintestinal disease.	Prepared microscope slides of trophozoites and cysts of *E. histolytica* may be compared with those of *Entamoeba coli* and *E. nana.*
18.22 Mastigophora, the Flagellates			
18.221 *Giardia lamblia*	*G. lamblia* is the most frequently recognized pathogenic protozoan in waterborne disease outbreaks and in tourists returning to the United States from abroad. Large numbers of *G. lamblia* attached to the wall of the small intestine may cause irritation and low-grade inflammation. Diarrhea may occur, especially in children, but asymptomatic cases are more common. The organisms are acquired by ingestion of cysts from contaminated food or water.	Diagnosis is by demonstration of cysts or trophozoites in feces. Metronidazole is the treatment of choice.	Prepared microscope slides of trophozoites and cysts of *G. lamblia* may be compared with those of *Chilomastix mesnili,* a nonpathogenic flagellate.
18.222 *Trichomonas vaginalis*	Three species of trichomonads are found in humans. The only pathogenic species, *T. vaginalis* may cause vaginitis in the female and urethritis in the male. *T. vaginalis* is transmitted by sexual contact.	Prevention is by attention to personal hygiene and detection and treatment of infected females and contacts. Diagnosis is made by microscopic examination of exudates or urine. No cyst exists. Metronidazole is the treatment of choice.	
18.223 Trypansoma	*Trypanosoma gambiense* and *T. rhodesiense* are causes of African trypanosomiasis (sleeping sickness) in humans. The organisms are transmitted by the bite of the tsetse fly. A mild systemic illness gradually	Trypanosomes, which spend part of their life cycle in vertebrates and part in invertebrates, may be found in the bloodstream of infected humans and other vertebrates. Diagnosis is by identification of the trypano-	

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	progresses into encephalitis. As cerebral damage increases, sleep becomes continuous and develops into a coma and death. Chaga's disease (American trypanosomiasis) is caused by *T. cruzi* and is transmitted by the treatomine (cone-nosed) bugs. It is the leading cause of cardiovascular death in South America but is generally not found as far north as the United States.	somes in blood, lymph glands, or spinal fluid. Treatment varies with stage of disease and is often not successful.	
18.224 *Leishmania*	Infection by members of the genus *Leishmania* results in various diseases which include visceral and/or dermal lesions. All species are transmitted by the bite of sand flies. *Leishmania* are not normally found in the United States.	An important example of leishmaniasis is kala-azar. Diagnosis is made by finding the organism in infected tissue or by serology. Treatment is varied and not often successful.	
18.23 Ciliata, the Ciliates			
18.231 *Balantidium coli*	*Balantidium coli* is the only ciliate which is parasitic for humans. The incidence is low, and many of these cases are asymptomatic. Infection is acquired by ingestion of cysts from contaminated food or water. Symptoms include alternating diarrhea and constipation.	The large intestine is invaded by the multiplying organisms which form nests that produce ulcers and abscesses. Oxytetracycline is the drug of choice.	
18.24 Sporozoa	The sporozoa of major medical importance are the four plasmodial species: *Plasmodium vivax*, *P. malariae*, *P. falciparum*, and *P. ovale*, all of which cause malaria.		
18.241 Plasmodia	Malaria is initiated by sporozoites from the salivary glands of infected female *Anopheles* mosquitoes. The sporozoites are injected into the bloodstream during the bite of the mosquito. The organisms undergo an exoerythrocytic cycle in the liver followed by an intraerythrocytic cycle in the blood as well as a sexual cycle which is completed in the mosquito. The major features of infection include anemia, spleen, and liver enlargement and a classical fever pattern. *P. falciparum* is the most important species.	In malaria, the sudden rupture of parasitized erythrocytes with release of parasites is responsible for the characteristic cycles of rising temperature followed by chills and 1 or more days free from symptoms. Pathology is caused by the destruction of erythrocytes, blockage of capillaries in the viscera, and damage to the liver due to a lack of oxygen. Diagnosis is by microscopic examination of blood smears. The disease is commonly treated or prevented with chloroquin or atabrine and by other synthetic drugs.	Prepared microscope slides containing the four species of plasmodia may be studied.

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18.242	*Toxoplasma gondii*	*T. gondii* is an obligate intracellular parasite of the reticuloendothelial system. The parasite is coccidian, and sexual reproduction occurs in the intestine of cats. A tissue phase is found in many animals (humans, cows, pigs, sheep, chickens, and rats) which have acquired the infection from ingesting fecal matter from cats or meat from infected animals. Toxoplasmosis in adults may be asymptomatic, chronic, or more rarely acute. This organism may be transmitted from an infected mother to the fetus leading to birth defects, abortion, or stillbirth.	Prevention is by thorough cooking of meat and careful handling of cat litter boxes, especially by pregnant women. Diagnosis is usually by serological methods, but microscopic examination of appropriate tissue may reveal organisms. Treatment is with pyrimethamine and sulfonamides.	Prepared microscope slides of tissue containing *T. gondii* may be studied.
18.3	General Features of Helminth Infection			
18.31	Transmission of Helminths	Transmission of helminths may be direct by ingestion of ova or by penetration of the skin by larvae. In certain cases, transmission is by eating improperly cooked meat containing larval forms. Some larvae may also penetrate human skin after undergoing a developmental cycle in snails and then becoming free-living larvae.	Many ova are infective for a new host after the ova have undergone some development outside of the body. Other ova are infective immediately. Historically, the religious prohibitions against eating pork were laid down because of the illnesses which were produced after eating incompletely cooked infected meat.	
18.32	Pathogenesis of Diseases Produced by Intestinal Helminths	Injury may be produced by the adults in the intestinal tract where irritation or penetration of the intestinal wall may take place or where attachment of the helminth may open the way for bacterial infection. Larvae may produce local or generalized reactions during their invasion and migration through the host's tissue. Localized reaction depends on the degree of sensitization of the host to products of the parasite.		
18.33	Symptoms of Intestinal Helminth Infection	Symptomatology of helminth infections depends upon the species, number, and locations of these parasites, the amount of migration of the organisms through the tissues, and the degree of sensitization of the host to the parasite. Chronic and repeated helminth infestations may result in anemia,		

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		intermittent abdominal symptoms, signs of pneumonia during larval migration, and lowered general health.		
18.4	Medical Helminthology			
18.41	Nematodes, the Roundworms			
18.411	*Ascaris lumbricoides*	The adult ascaris is the largest of the human intestinal roundworms. It may reach 20 cm in length. Ascariasis is the most common helminthic infestation of humans. It occurs when ingested eggs hatch in the small intestine, enter the bloodstream, and reach the lungs, where they are coughed up and swallowed. Adults reach maturity in the intestinal tract. Symptoms may include abdominal pain and allergic reactions to the pulmonary cycle.	Transmission is by the fecal-oral route, with infestation most prevalent in young children. Eggs leave the body of the host in feces, develop in soil, and become infectious after 10 to 14 days. Large numbers of adult worms may cause obstruction of the small intestine. Diagnosis is made by finding eggs in the stool. Treatment is with piperazine citrate and other drugs.	
18.412	*Enterobius vermicularis* (Pinworms)	These small roundworms (2 to 3 mm) are inhabitants of the cecum. Infection is common in temperate climates and is not limited to lower socioeconomic groups. At night, the female emerges from the anus and lays her eggs in the perianal region. Itching develops around the perianal area which leads to reinfection. The eggs may be spread throughout the house by contaminated bedclothes, fingers, and other objects.	Enterobiasis is extremely prevalent in groups of young children and their households. Infection is self-limiting in the absence of reinfection. Diagnosis is made by microscopic examination of cellophane tape which has been pressed around the perianal region. Treatment is with pyrivinium pamoate, piperazine, or pyrantel pamoate. Generally an entire household must be treated.	
18.413	*Necatur americanus*	The adult *N. americanus*, the New World hookworm, is approximately 1 cm long. It attaches to the mucosa of the small intestine by two pairs of chitinous plates in its mouth. Eggs leave the host in feces and develop to yield larvae in 24 to 48 hours. Larvae penetrate through the skin to the blood vessels and lungs where they are coughed up and swallowed. Maturation takes place in the small intestine. Infestation with a small number of worms may be asymptomatic. In chronic infestation with a large number of	Hookworm infection may be prevented by proper sanitary measures and by wearing shoes in infested areas. Diagnosis is made by demonstrating eggs in stool specimens. Treatment of hookworm infections is usually not necessary. An iron-rich diet helps to prevent symptoms. *Ancylostoma duodenale*, the Old World hookworm, is common in southeast Asia. *A. braziliense* and *A. caninum* are parasites of dogs and cats which occasionally infect humans. Creeping eruption or cutaneous larva	

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		worms, 100 to 200 ml of blood per day may be removed resulting in iron-deficiency anemia.	migrans caused by these animal parasites is produced when the larvae burrow under the human skin producing irritating tunnels. Diagnosis is made by observing the tracks in the skin.	
18.414	*Strongyloides stercoralis* (Threadworm)	The adult worms of *S. stercoralis* parasitize the duodenum or jejunum of humans. The eggs, passed by the fertilized female, hatch in the intestinal mucosa, and the larvae leave the body in the feces. Infection is acquired by penetration of the larvae into the skin of the new host followed by passage through the bloodstream to the lungs, where they are coughed up and then swallowed. Diagnosis is made by finding the larvae in the feces.	The life cycle of *Strongyloides* is very complex; different life cycles may occur during a single infestation. Strongyloidiasis is characterized by watery, mucoid diarrhea. Treatment is with thiabendezole.	
18.415	*Trichuris trichiura* (Whipworm)	Adult worms are found in the cecum. The eggs are passed in the feces to the soil where embryonic development takes place. Infected eggs are ingested by the new host and develop into the adult stage in the intestines. Trichuriasis is a common disease among children of tropical countries and is often asymptomatic. In heavy infections, prolapse of the rectum, loss of weight, vomiting, diarrhea, and dysentery occur due to the worms penetrating the mucosa.	Prevention is by sanitary waste disposal and washing of hands. Diagnosis is made by examining feces for unsegmented lemon-shaped eggs with plug-like ends. Trichuris infections may be treated with mebendazole.	Prepared microscope slides and preserved material may be studied to illustrate eggs and adults.
18.416	*Trichinella spiralis*	Humans become infected with *T. spiralis* by eating insufficiently cooked meat of flesh-eating mammals or certain domestic animals (pigs) which contain larvae encysted in striated muscle. Worms from digested cysts develop in the intestine and produce larvae which penetrate the mucosa of the small intestine and are disbursed by the blood. They invade striated muscle primarily of the diaphragm, arms, and legs. Most cases are asymptomatic; however, symptoms may occur depending on the particular tissue invaded and severity of infection. An acute inflammatory reaction may occur around parasitized muscle fibers.	The domestic animal cycle depends upon the feeding of raw garbage containing infected table scraps to swine. Diagnosis of trichinosis is by intradermal testing, muscle biopsy, or serological testing. There is no specific treatment, but steroids may provide symptomatic relief.	Prepared microscope slides of encysted larvae may be observed.

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18.42	Cestodes (Tapeworms)			
18.421	*Taenia solium* (Pork Tapeworm)	The adult of *T. solium* is found in the upper part of the jejunum of humans where it attaches by means of hooks on its scolex. Eggs or proglottids are passed in the feces and eaten by hogs. The eggs hatch in the intestinal tract of the hog, then penetrate the intestinal wall and migrate to muscles and other tissues. Infection of humans occurs from eating undercooked pork in which the larvae are encysted. Worms are 2 to 4 meters in length and have less than 1,000 proglottids.	Diagnosis is made by finding proglottids or eggs in feces or on swabs from the perianal region. Occasionally, encysted larvae may develop in humans. Eggs in the feces may be swept back into the duodenum by reverse peristalsis. The eggs hatch and the larvae migrate to muscle tissue or brain tissue where they encyst and cause disease.	Prepared microscope slides and preserved fecal matter containing ova may be observed. Preserved proglottids or entire adults may also be studied.
18.422	*Taenia saginata* (Beef Tapeworm)	Humans are the definitive host of *T. saginata*. Intermediate hosts are herbivores, with cattle being the most important. Grazing cattle are infected by eating ova or proglottids contained in contaminated feces of humans. The eggs hatch in the intestine, larvae penetrate the intestinal wall and migrate to muscles, where they become encysted. Infection occurs when the cyst is ingested in undercooked meat. Worms are 4 to 10 meters in length and have approximately 2,000 proglottids. *T. saginata* however produces little toxic, irritative or allergic reaction.	Diagnosis is made by finding eggs or proglottids in feces or on swabs from the perianal region. Treatment is with niclosamide. If the scolex is not passed following treatment, more proglottids may develop.	Prepared microscope slides and preserved fecal matter containing ova may be observed. Preserved proglottids or entire adults may also be studied.
18.423	*Diphyllobothrium latum* (Fish Tapeworm)	*D. latum* is the largest tapeworm of humans reaching a length of more than 10 meters. There are more than 3,000 proglottids per tapeworm and 1 million eggs may be excreted by the tapeworm each day. Infection occurs from eating raw or undercooked freshwater animals. Eggs are passed in the feces and hatch in fresh water. If the intermediate host (fish, crustaceans) is ingested, larvae develop to the adult stage in the ileum.	The fish tapeworm is found in cold, freshwater lakes. In areas where untreated sewage is allowed to enter fresh water, infection of fish and crustaceans occurs.	
18.424	*Hymenolepsis nana* (Dwarf Tapeworm)	*H. nana* is the most common tapeworm of humans, generally occurring in children	Diagnosis is by finding the eggs in feces.	

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		in tropical climates. No intermediate host is required. Ingested eggs hatch in the small intestine, penetrate the villi, and later break out into the lumen and attach to the mucosa. Light infections show no symptoms, whereas heavier infections produce abdominal pain and diarrhea.		
18.43	Liver Flukes	Liver flukes are flattened and monoecious, possess oral and ventral suckers, and live in the bile ducts of the liver. Major genera include *Clonorchis*, *Opisthorchis*, and *Fasciola*. Life cycles involve snails as intermediate hosts and mammals including humans as definitive hosts. Fish may also act as additional intermediate hosts. Flukes are endemic in the Far East where fish ponds are fertilized with human feces.	Most cases are asymptomatic; however, heavy infection results in abdominal pain and diarrhea. Diagnosis is made by finding eggs in feces. In many Far East countries, raw fish are traditionally eaten and are a common source of infection. Liver flukes may live for 20 years.	
18.44	Schistosoma (Blood Flukes)	Schistosomiasis is a disease caused by members of the genus *Schistosoma* which live in the blood vessels. Eggs are passed in urine and/or feces. The eggs are ingested by specific snails which serve as intermediate hosts. The free-swimming larval stage is infective by penetrating the human skin. Symptoms may vary from a transient rash to acute illness and possible death.	Diagnosis is made by finding eggs in feces and/or urine. Schistosomiasis is found in Africa, South Asia, and tropical America.	
19.0	**Diagnostic Microbiology**			
19.1	Diagnostic Bacteriology	Diagnostic bacteriology is primarily concerned with the identification of organisms as they relate to infectious disease and to the relative resistance or susceptibility of pathogenic bacteria to antibacterial agents.		
19.2	Transport	Specimens should be taken from appropriate body sites by using aseptic techniques and transported to the laboratory as quickly as possible in appropriate transport media.	Examples of specimen sources include throat, nasopharynx, wounds, sputum, blood, cerebrospinal fluid, urine, feces, and tissue biopsies.	
19.3	Sterile Body Sites	Many body sites or tissues such as blood and cerebrospinal fluid are normally devoid		

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		of microorganisms so that recovery of microorganisms from these areas is significant.		
19.4	Normal Flora	Many parts of the body have normal microbial flora so that microorganisms cultured from these areas must be considered in this light.	*Escherichia coli* is normally found in the stool but is a common urinary tract pathogen.	Throats may be swabbed and specimens cultured on blood agar plates.
19.5	Isolation	To identify bacteria, it is generally necessary to streak the specimen on appropriate semisolid agar in a petri dish and isolate colony-forming units.	It is difficult and usually impossible to identify bacteria if they are not in pure culture.	Pure and mixed cultures of bacteria streaked for isolation should be studied.
19.51	Initial Information on Bacteria	The Gram reaction, cellular morphology and arrangement, and colonial morphology and growth on selective and differential media provide valuable information toward identification of many bacteria.	Speed and accuracy of organism identification is necessary for patient care.	Colonial characteristics of bacteria grown on semisolid media in petri dishes may be studied. Gram stains should be performed.
19.52	Bacterial Identification	The ability to grow and reproduce in various biochemical media, the end products of such growth, susceptibility to certain antibiotics, and reactions with specific antibody are common methods in bacterial identification.	Identification of anaerobic bacteria is aided by the use of gas-liquid chromatography for detection of characteristic fatty acid end products.	Many commercially produced systems are used in clinical laboratories for the identification of *Enterobacteriaceae*, nonfermenters, and yeasts. These systems might be obtained from clinical laboratories for demonstration.
19.6	Antibiotic Susceptibility	When a pathogenic bacterial strain is isolated from a patient, antibiotic susceptibility patterns of that organism are often considered more important than its identification. A bacterial isolate is generally subjected to a panel of antibiotics to determine its susceptibility or resistance to each drug.	The susceptibility pattern of a bacterial isolate is often determined and reported along with a brief description of the organism (e.g., gram-negative bacillus) before the organism is identified.	An experiment showing the effects of various antibiotic disks dropped on a lawn of bacteria growing on semisolid agar in a petri dish may be performed.
19.7	Parasite Identification	Diagnostic laboratory parasitology primarily involves searching for the adult organisms, eggs, cysts, or trophozoites in patient specimens. Material is rarely cultured.	Some serological procedures are available which aid in the diagnosis of certain parasitic diseases such as amoebiasis and trichinosis. Toxoplasmosis is primarily diagnosed by serological means.	Prepared microscope slides of parasitic protozoans may be studied.
19.8	Fungus Identification	Detection and identification of fungi in the diagnostic laboratory are based primarily on recognizable macroscopic and microscopic characteristics. Growth of fungi in	Serological techniques are available as an aid in the diagnosis of certain fungal infections. However, serological cross-reactions among some of the fungal agents make in-	Projection slides illustrating macroscopic and microscopic fungal characteristics may be studied.

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		clinical specimens often requires many weeks.	terpretation difficult. Diagnosis of cryptococcal meningitis is aided by the immunological detection of the cryptococcal antigen in cerebrospinal fluid.	
19.9	Virus Identification	Few clinical laboratories are equipped to isolate and identify a virus. Growth must take place in living tissue, and identification is usually made from this tissue based on specific antibody reactivity or characteristic cytopathogenic effects of a given virus on specific cell cultures, embryonated eggs, or laboratory animals.	For the most part, laboratory evidence of viral infection is obtained by serological means rather than by virus isolation. The time involved in viral isolation and identification can vary from a few hours (herpes simplex virus) to 3 to 5 weeks (cytomegalovirus). Also, serological evidence of viral infection requires submission of an acute and convalescent blood specimen (usually 10 to 14 days apart) to demonstrate a significant rise in antibody titer.	Projection slides showing cytopathic effects in tissue or embryonated eggs may be shown.
20.0	**Diagnostic Immunology**			
20.1	Serology	Serological tests attempt to detect specific antigens or antibodies in any body fluid or tissue of a patient. The results are of clinical importance in the identification of infectious agents, self and fetal antigens and their corresponding antibodies. In all serological testing, the identity of one component, antigen or antibody, is known.		
20.11	Identification of Antigens	An unknown antigen can be identified or typed by means of known antibody.	An organism may be identified as a member of the *Salmonella* genus by the use of specific antiserum.	
20.12	Identification of Antibody	The presence or absence of antibody and the titer (level) of antibody can be determined by employing known antigen in the antigen-antibody reaction.	Serial dilutions of a patient's serum may be tested with known antigen. A low titer may suggest that a patient has had previous infection or immunization with that organism. A rise in titer, when acute and convalescent serum samples are compared, suggests that active infection is or recently was present.	
20.2	Antigen-Antibody Reactions			

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20.21	Valence of Antibody	Valence is the number of antigenic determinants or sites with which one antibody molecule can combine. Immunoglobulin G (IgG) antibodies are bivalent (two sites), whereas IgM has a valence of 5 to 10.	Electron micrographs show antibodies attached to antigens by two attachment sites in the case of IgG and two or more for IgM.	A diagram showing sites for antigen binding in IgG and IgM molecules may be studied.
20.22	Valence of Antigen	Antigens are multivalent, i.e., have many antibody-combining sites.	Electron micrographs show that a microorganism may have many antibodies attached to it.	
20.23	Lattice Effect	When multivalent antigens and bivalent antibodies combine, they link in a lattice-type formation.	The lattice effect is dependent upon pH, ionic strength, and temperature.	A diagram or drawing of the lattice effect may be studied.
20.3	Serological Tests			
20.31	Agglutination Test	The reaction between antibody and a particulate antigen is referred to as an agglutination reaction. The antigen may consist of suspensions of organisms, erythrocytes, or uniform particles such as latex beads which have antigen adsorbed onto them.	Agglutination reactions are dependent upon pH, ionic strength, and temperature.	Projection slides of agglutination tests and reactions may be studied.
20.311	Basis of Test	Particulate antigens mixed with specific antibody clump due to the antibody holding them together.	Salmonella typing may be carried out by either slide or test tube agglutination procedures.	Agglutination reactions such as those for the detection of rheumatoid factor are commercially available and may be performed.
20.312	Hemagglutination	Hemagglutination is the ability of certain substances to attach to (adsorb) the surface of erythrocytes resulting in a clumping of the red cells. Influenza virus hemagglutinates erythrocytes and can be detected and quantitated by this method.	Many other viruses are also able to agglutinate erythrocytes. This phenomenon is not dependent on the presence of antibody.	
20.313	Hemagglutination Inhibition	Specific antiserum against the influenza virus will combine with its antigen (virus) and prevent the virus from attaching to the erythrocytes and thus prevent hemagglutination. This is known as hemagglutination inhibition.	Hemagglutination inhibition testing may be used to identify an unknown virus or specific antibody to a known virus.	
20.314	Blood Typing	Blood groups are systems of different antigens on the surfaces of erythrocytes. The most important are the A, B, O, and Rh	A person who has group A antigens on his erythrocytes (type A blood) will also have antibodies to group B antigen and con-	Typing of students' blood may be performed with commercially available typing sera.

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	systems which may be involved in transfusion reactions and hemolytic disease of the newborn.	versely, type B blood contains antibodies to group A antigens. Therefore, transfusion of blood from a type A donor into a type B recipient or vice versa would result in a tranfusion reaction.	
20.32 Precipitation	The reaction between specific antibody and soluble antigen is referred to as a precipitation reaction. The antigen-antibody complex becomes insoluble.	Qualitative and quantitative tests for specific serum proteins such as albumin are often performed by precipitation testing.	Precipitation testing using albumin or any protein and its specific antibody may be conducted by using capillary tubes or test tubes.
20.321 Gel Diffusion	Gel diffusion is a precipitation reaction in which the antigen and antibody reactants are supported by an agar matrix. The reactants diffuse toward one another and form a detectable insoluble complex recognized as a precipitation line.	Various methods involving gel diffusion have been developed of which double diffusion in agar is a characteristic example.	The reaction between albumin and antialbumin may also be detected by gel diffusion. Filter paper disks saturated with the reactants are placed 1 cm apart on an agar gel surface and allowed to diffuse over night.
20.33 Complement Fixation	The binding (fixation) of complement occurs following the reaction between an antigen and its specific antibody. The fixation of complement can be used as a test to detect and semiquantitate antibodies in a patient's serum. This test is most commonly used to detect antibodies to viruses.		
20.34 Immunofluorescence Microscopy	Antigen or antibody is coupled to a fluorescent dye and then reacted with the unknown. The reaction is generally carried out on a microscope slide. A specialized microscope is used for observation. If fluorescence is seen, a specific antigen-antibody reaction has taken place.	The presence of certain antibodies in serum, identification of microorganisms, and detection of antigens in tissue sections is often determined via immunofluorescence microscopy.	
20.35 Radioimmunoassay (RIA)	Radioimmunoassays are useful in measuring the serum levels of many hormones, drugs, and other biological materials. The method is most often based on competition for specific antibody between radioactively labeled known material and unlabeled unknown material.	Radioimmunoassay is very sensitive and capable of detecting substances in nanograms per milliliter.	
20.36 Enzyme-Linked Immunosorbent Assay (ELISA)	ELISA is analogous to radioimmunoassay or immunofluorescence except that the antibody is conjugated to an enzyme. Detec-	ELISA testing approaches the sensitivity of RIA without the drawback of using radioactive materials.	

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		tion of an antigen-antibody reaction is then dependent on a color reaction resulting from the reaction of the enzyme with its substrate. The substrate is chosen so that it is colorless initially and, on degradation by the enzyme, yields a colored product whose intensity can be measured spectrophotometrically.		
20.4	Skin Tests Involving Immediate Hypersensitivity (Atopic Allergy)	In atopic allergy testing, the patient is injected intradermally with a series of antigens (allergens such as pollen, danders, etc.) to determine the ones to which he is sensitive. If specific antibody (IgE) is present, a localized reaction occurs at the injection site within minutes.	Genetic factors seem to predispose individuals to atopic allergies. Approximately 10% of the population suffers from asthma, hay fever, and other atopic allergies.	
20.5	Skin Tests Involving Delayed Hypersensitivity	Delayed hypersensitivity skin testing involves the intradermal injection of certain antigens to demonstrate cutaneous hypersensitivity. Since these tests involve a cell-mediated (T-cell) response, a localized reaction at the injection site occurs within 24 to 72 hours.	Delayed hypersensitivity skin testing is used to demonstrate hypersensitivity to tuberculosis, systemic fungal diseases, and poison ivy. It is also used in the assessment of immunocompetence.	
20.6	Detection of Cells in the Immune Response and Their Function	The amounts and the function of lymphocytes, granulocytes, and macrophages are important in determining the immunocompetence of a host. There are a variety of methodologies by which the amounts and function of these cells can be determined.	Lymphocytes which bind sheep erythrocytes to form rosettes are T cells. This marker is used to identify and quantitate T cells. Lymphocytes with surface immunoglobulins [B cells] can be identified and quantitated by immunofluorescence.	
21.0	**Epidemiology**			
21.1	Epidemiology	Epidemiology is the study of the factors which determine the frequency and distribution of infection and disease in a given population.	Knowledge of the distribution of a disease within a population can be used to identify the etiological agent and to design strategies for its prevention and control.	
21.11	Sporadic	Some diseases may be present in a community with only a few cases becoming clinically evident from time to time. These cases are said to occur sporadically.	Virus diseases such as mumps may occur sporadically in school age children.	
21.12	Endemic	A disease is said to be endemic if it is continuously present in a given population at a low level of incidence.	Measles is an example of an endemic disease in the United States.	

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21.13	Epidemic	An epidemic is a rapidly progressing outbreak of an infectious disease through a population of susceptible individuals. An endemic disease which significantly increases in incidence within a given period of time can become epidemic.	No specific number of cases can be used to identify an epidemic. One hundred cases of bacillary dysentery per 100,000 persons in the United States would be considered an epidemic, whereas the same incidence in the Far East might not be considered unusual or may even be considered as endemic. The possibility of a particular organism producing an epidemic depends in part on the number of individuals in the population who have immunity to the organism either from previous infection or from immunization. The genetic background of the population, the virulence of the particular strain of the pathogen, and the intensity of exposure of a given group of individuals in the population are all factors involved in determining whether a disease becomes epidemic.	Weekly epidemiological reports of communicable diseases within each state may be obtained from the local health department.
21.14	Pandemic	A pandemic is an epidemic of unusally great proportions often involving more than one country or continent.	Influenza is a classical example of an infectious agent that has caused pandemics in the past.	
21.15	Recognition of Epidemics	A continuing survey of the morbidity rate (number of cases per 100,000 persons) and mortality rate (number of deaths per 1,000 cases) of a given disease will indicate an epidemic if either rate suddenly increases in frequency.	Statistics are kept by health departments as to the seasonal incidence and localities involved in the spread of disease.	
21.16	Reservoir of Infection	Reservoirs of infection include carriers or active cases, soil and water which might harbor organisms, or animals which might harbor an infectious agent which is transmissible directly or indirectly to humans.	The reservoir of a disease-producing infectious agent is the sum of all the potential sources of that infectious agent.	
21.17	Zoonotic Infections	Zoonoses are infections of animals which can be transmitted directly or indirectly to humans.	Rabies is primarily a disease of skunks and bats which act as a reservoir with potential infection to humans.	
21.2	Transmission of Disease	Factors determining the method of transmission of disease are the means by which the microorganisms leave the body (the portal of exit), survival of the microorganisms	Portals of exit and entry include direct contact between individuals, the respiratory route, the fecal-oral route, and inoculation by animals, insects, or instruments.	

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		while outside the body, and the method of entering the new host (portal of entry).		
21.21	Diseases Transmitted by Direct Contact	Diseases which require direct contact for transmission are caused by microorganisms that require a living host and which have a low resistance to the environment outside of the host.	The fastidious nature of these organisms show a high degree of adaptation of the parasite to its host.	
21.211	Sexually Transmitted Disease (STD), or Venereal Diseases (VD)	VD are transmitted almost solely by sexual contact or by routes which include direct contact from a lesion of one person to the skin or mucous membranes of another.	Syphilis and gonorrhea are sexually transmitted diseases. The necessity of a close relationship between these parasites and their host is shown by their inability to withstand environmental conditions outside of the host.	Films about venereal diseases may be shown to offer an understanding of epidemiological principles as well as information about the diseases.
21.22	Diseases Transmitted by Air	Airborne transmission of disease is caused by inhalation of droplets of sputum or saliva from an infected host or inhalation of dust which contains spores from an organism growing in soil. Air-borne (respiratory) transmission is the most common method by which infectious agents are transmitted in humans.	The common cold and influenza are two examples of diseases transmitted by droplets and which use the respiratory tract as the portal of entry and the portal of exit.	
21.221	Dust-Borne Organisms	Fungi which cause systemic infections grow in the soil and produce spores in large numbers which are inhaled with circulating dust.	Blastomycosis, histoplasmosis, coccidioidomycosis, and cryptococcosis are examples of systemic mycoses transmitted by this method.	
21.23	Food-Borne and Waterborne Diseases	Organisms which leave the body in excrement may find their way directly into water and indirectly into food by way of foodhandlers or flies. Bacteria, viruses, and many intestinal protozoa are infective if consumed by a new host. Some helminths, however, complete part of their life cycle in the water or in another organism before becoming infective for a new human host.	Organisms such as intestinal viruses may only survive in water, but bacteria such as salmonellae may multiply in great numbers in food such as meat and egg products.	Food-handler laws and Environmental Protection Agency regulations may be discussed.
21.231	Food Infection Versus Food Intoxication	Disease produced by microorganisms may be either infectious (the organisms attack the cells of the host) or intoxications (the organisms do not have to be present but	Boils caused by *Staphylococcus aureus* are an example of an infection, whereas food poisoning caused by enterotoxins produced by *S. aureus* is an intoxication.	

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		toxins produced by the microorganisms may cause the disease).		
21.24	Diseases Transmitted by Inoculation	Inoculation can occur by insect and animal bites, by puncture wounds, or, more rarely, by needle and syringe.	Direct inoculation of microorganisms by arthropods may be necessary to transmit certain organisms which have no other portal of entry to the host. Other organisms require damaged tissue to become established.	
21.241	Transmission by Arthropods	Many arthropods (insects such as mosquitoes, ticks, and fleas) may act as vectors by transmitting infectious agents from one host to another (animal to human or human to humans).	Rocky Mountain spotted fever and malaria are examples of diseases transmitted by arthropods. Control of animal reservoir hosts and elimination of arthropod vectors help control the spread of certain diseases.	
21.242	Transmission by Animal Bite	A small number of diseases are transmissible to humans by animal bites. Rabies is the classical example.	The rabies virus is present in the salivary glands as well as other tissues of infected carnivores. The virus in the saliva is able to establish itself only because the bite produces abraided tissue.	
21.243	Transmission by Puncture Wound	Gas gangrene and tetanus are caused by organisms which gain entrance to the body through a deep wound. The diseases are caused by the exotoxins produced by these anaerobic sporeforming bacilli.	If an individual has been previously immunized or is given a prophylatic injection of antitoxin, tetanus may be prevented.	
21.244	Transmission by Artificial Inoculation (Needle and Syringe)	Although any infectious agent can be transmitted accidentally by a contaminated needle puncture, hepatitis B virus (HBV) is the most significant. Hospital personnel, homosexuals, and drug addicts are at greatest risk.		
21.3	Nosocomial Infections (Hospital Acquired)	Hospital-acquired infections can be transmitted to patients by direct contact with other patients or hospital personnel, by air, food or water, or by contact with contaminated fomites (inanimate objects) such as syringes, needles, or bed clothing.	These diseases may be especially severe because of the virulence of the organisms and/or the lowered resistance of the patient. Gram-negative rods are a frequent cause of nosocomial infections.	
21.4	Quarantine	Quarantine is the isolation of individuals with highly contagious disease to minimize or eliminate spread of this disease to others.	Some states permit state health departments to close schools, theaters, or other large gathering places to avoid epidemics.	

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21.5 Immunization	Prevention or control of epidemics can be facilitated by active immunization of susceptible populations. Many infectious agents capable of causing epidemics such as polio and measles are now controlled by immunization programs.		

Section III: Microbial Physiology

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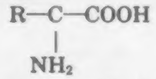
1.0 **Biochemicals**	To understand the nutrition and metabolism of microorganisms, it is necessary to have some basic knowledge about the chemicals that are involved in life processes.	For all of this section, a textbook of biochemistry is advised as a reference.	For all of Section 1.0, sets of molecular models may be used for three-dimensional visualization of these compounds.
1.1 Small Molecules	All living material is assembled from small organic molecules.	A number of texts in microbiology have chapters giving information about these compounds.	
1.11 Sugars	Sugars have the general formula $(CH_2O)_n$. They are water soluble.	Sugars are carbohydrates. The name carbohydrate is derived from the fact that for each carbon atom there are usually two hydrogens and one oxygen (the same ratio as in water).	Various chemical tests can be used to detect sugars. The Benedict test is in many introductory biology laboratory manuals.
1.111 Monosaccharides	Some important monosaccharides are the three-carbon sugar glyceraldehyde $(C_3H_6O_3)$, various five-carbon sugars (pentoses), and a number of six-carbon sugars (hexoses).	Glucose, fructose, and galactose are commonly occurring six-carbon sugars. Common five-carbon monosaccharides are ribose and deoxyribose. The differences in the arrangement of hydrogen (H) and hydroxyl (OH) groups around carbons in the central chain account for the different six-carbon sugars.	
1.112 Disaccharides	Disaccharides are composed of two monosaccharides covalently bonded together. Two common disaccharides are sucrose and lactose.	Sucrose is composed of one molecule of glucose and one molecule of fructose; lactose has one molecule of glucose and one of galactose.	
1.113 Substituted Sugars	Substituted (modified) sugars are important in biological structures.	Some of the ways that simple sugars can be modified are removal of hydroxyl groups, e.g., deoxyribose; addition of nitrogen-containing groups, e.g., *N*-acetylglucosamine; and oxidation of one of the hydroxyl groups, e.g., gluconic acid.	Muramic acid, a modified sugar found only in procaryotic cell walls, has been used as a sensitive indicator of the presence of procaryotic organisms in various natural samples.
1.12 Amino Acids	Amino acids have an amino group (NH_2) and a carboxyl (COOH) group. There are about 20 amino acids important in protein structure. These units are building blocks of proteins.	A basic formula is  $R{-}\underset{\displaystyle NH_2}{\underset{\vert}{C}}{-}COOH$ where R is an organic radical. These organic radicals have a wide variety of chemical properties. Depending on the R-group property, an amino acid may be acidic, neutral, or basic, and more or less water soluble.	Some microorganisms synthesize all of the amino acids they need, but others require some preformed amino acids. The fact that some microorganisms require amino acids for growth has been the basis of quantitative assays of amino acids with microorganisms (bioassay).

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			Examples of amino acids are: glutamic, R = $C_2H_4COO^-$; lysine, R = $C_4H_8NH_3^+$; glycine, R = CH_3.	
1.13	Fatty Acids	These are chains of carbon (C) with attached hydrogen ending with a carboxyl group (COOH). They range in length from two carbons up to 22 or more carbons.	Volatile short chain fatty acids, for example acetate (2C), butyrate (3C), and propionate (4C) are produced by fermenting microorganisms as fermentation products. Longer-chain fatty acids are synthesized by essentially all microorganisms for use in membrane lipids.	Growth of some fungi can be inhibited by fatty acids. For example, some athlete's foot remedies contain fatty acids. The natural fatty acids on skin inhibit bacterial growth. Volatile fatty acids produced by anaerobes are useful in identification of these organisms.
1.14	Nucleotides	Nucleotides are composed of a nitrogenous base (often a purine or pyrimidine) linked to a five-carbon sugar (ribose or deoxyribose) which has one to three phosphate groups on it.	Nucleotides function as subunits of nucleic acids (Microbial Genetics, Subtopic 2.11). Also some nucleotides, e.g., adenosine triphosphate, function as energy carriers (Microbial Physiology, Subtopic 5.16).	
1.2	Macromolecules	These are composed of smaller molecules covalently bonded together in an orderly and repeating arrangement.	The small molecules are linked together by enzymatic processes which eliminate water (condensation or dehydration reactions).	
1.21	Polysaccharides	These are composed of monosaccharides linked together.	Some biologically important polysaccharides are starch, cellulose, glycogen, and dextrans—all composed of glucose units linked together in various ways. Other polysaccharides are composed of more than one type of monomer (Microbial Physiology, Subtopic 1.33).	You can demonstrate that starch is made of monosaccharides by adding some saliva (which contains the enzyme amylase) to a starch solution and testing for monosaccharides released as the starch is broken down.
1.22	Proteins	These are composed of large numbers of amino acids linked together according to a genetically determined pattern (Microbial Genetics, Subtopic 2.31). The bond between each amino acid in the sequence is called a peptide bond.	Peptide bonds are formed by linkage of the carboxyl (COOH) group of one amino acid to the amino (NH_2) group of another amino acid (Microbial Genetics, Subtopic 2.31).	
1.23	Nucleic Acids	These are composed of large numbers of nucleotides linked together. If the nucleotide sugar is ribose, the compound is ribonucleic acid (RNA). If the nucleotide sugar is deoxyribose, the compound is deoxyribonucleic acid (DNA).	(Microbial Genetics, Subtopics 2.1 and 2.2).	Charts showing the nucleotide composition and the helical structure of nucleic acids, as well as molecular models, are available. Nucleic acids are negatively charged and stain with basic dyes. If you look at methylene blue-stained preparations of pro-

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			tozoans or algae, the nuclei stain blue because of the negative property of the nucleic acid.
1.24 Lipids	Lipids are a heterogeneous group of compounds that are insoluble in water but are soluble in organic solvents such as ether. Phospholipids are important in membrane structure.	Fats and waxes are examples of simple lipids. Triglycerides, storage lipids, are composed of three fatty acid molecules linked to glycerol (a three-carbon compound). Phospholipids, essential compounds of membranes, contain a non-water-soluble portion (often a glycerol substituted with two long-chain fatty acids) and a water-soluble portion (a phosphate on the third carbon of glycerol and some charged groups attached to the phosphate). Sterols are more complex lipids found in large amounts in membranes of eucaryotic microorganisms. The procaryotic exceptions are the members of the genus *Mycoplasma* which incorporate sterols from the medium into their membranes.	
1.3 Macromolecular Complexes	Biological structures are specific assemblages of macromolecules.		
1.31 Lipopolysaccharides and Lipoproteins	These are composed of lipids linked with polysaccharides and lipids linked with proteins.	These compounds are structural components of bacterial cell envelopes and membranes.	
1.32 Ribosome (Ribonucleoprotein Complexes)	Ribosomes are complexes of RNA and protein molecules. They are the site of protein synthesis.	Ribosomes of procaryotic cells can be differentiated from those of eucaryotic cell cytoplasm by sedimentation in an analytical centrifuge. The Svedberg unit is a measure of sedimentation velocity. Procaryotic ribosomes sediment at 70 Svedberg units (70S) and eucaryotic ribosomes sediment at 80S. Mitochondria and chloroplasts of eucaryotic cells have 70S ribosomes.	There are antibiotics, e.g., streptomycin, that selectively inhibit procaryotic cells by binding to 70S ribosomes. There are other chemicals, e.g., cycloheximide, that inhibit eucaryotic cell growth by binding specifically to 80S ribosomes.
1.33 Peptidoglycan	Peptidoglycan consists of a polysaccharide backbone composed of two substituted monosaccharides (muramic acid and *N*-acetylglucosamine). Short chains of amino acids	Peptidoglycan is unique to procaryotic cells.	Most textbooks of microbiology have diagrams of the specific interrelationships of the amino sugars and amino acids under a discussion of bacterial cell walls. There are

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	connect the polysaccharide backbones together to give a rigid layer to the cell wall of bacteria.		antibiotics, e.g., penicillin, that inhibit the formation of the peptidoglycan cell wall of procaryotes while they are growing.
1.34 Membrane	The outer and inner membrane surfaces have the water-soluble ends of phospholipid molecules interspersed with protein molecules. The middle layer consists of the non-water-soluble fatty acid chains of the phospholipids. Interposed are some protein molecules that extend through the membrane.	Cell membranes have sites for active transport of nutrients into the cell. Active transport systems allow concentration of nutrients inside the cell. There are also enzymes that are active in energy metabolism associated with the membrane. Invaginations of the membrane provide more functional membrane surface in many phototrophic procaryotes (Microbial Physiology, Subtopic 6.0).	Some fungal membranes contain large amounts of sterols. Certain chemicals which react with sterols destabilize the membranes and thus can be used as antifungal agents. These chemicals are not active against procaryotic pathogens because these organisms generally lack membrane sterols. The inhibition of yeast but not bacterial growth by a compound like Nystatin can be demonstrated.
1.4 Cellular Composition	Microbial cells are composed of proteins, carbohydrates, nucleic acids, lipids, phosphates, and other materials. Seventy five to ninety percent of the weight of the microbial cell is water.	Composition of bacterial cells will vary depending upon the kind of bacteria and the growth medium. Very rough generalities of composition are as follows: Water 75 to 90% Dry weight of: Protein 35 to 50% Carbohydrate 2 to 25% Nucleic acids RNA 6 to 25% DNA 1 to 4% Peptidoglycan Gram positive cells 15 to 20% Gram negative cells 0.1 to 5% Lipopolysaccharide Gram negative cells 5% Inorganic (Phosphates) 0.9%	Wet weights and dry weights of a mass of concentrated bacterial cells can be determined to show the water content of the cell.
2.0 Nutrition	Nutrients are those materials taken from the environment from which cells draw energy and substances required for syntheses of cellular components. Because there is such a great diversity of microbiological types, there is a corresponding diversity in the types of nutrients required.	Materials from the environment must be able to enter the cell to be used as nutrients. Many organisms make from simple compounds all the amino acids, vitamins, and metabolic intermediates that they need for growth; others have extensive nutritional requirements. Some bacteria, such as the agents of leprosy and of syphilis, have never been cultivated on cell-free media.	Various media may be made to demonstrate the concepts that follow; care must be taken in media preparation. Some common problems and ways to avoid them follow. Mixtures of inorganic compounds may precipitate. Watch pH and chelating agents. Add only materials which have been dissolved in water, and add them in the order suggested in the formula being used. Many organic compounds interact at high

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			An example of the problems involved in obtaining the proper growth medium is the difficulty experienced in initial isolation of the agent of Legionnaire's disease. **Fraser, D. W. and J. E. McDade.** 1979. Legionellosis. Sci. Am. **241:** 82-99.	temperatures, i.e., autoclave glucose and KH_2PO_4 solutions separately. Many organic compounds are destroyed by temperatures attained in the autoclave. These should be sterilized by filtration.
2.1	Energy Requirements			
2.11	Chemotrophs	The chemotrophs are a class of microorganisms which obtain energy by the oxidation of chemical compounds or elements. There are two kinds of chemotrophs. Some oxidize inorganic elements or compounds, and others oxidize organic (carbon-containing) compounds. Bacteria oxidizing inorganic molecules are called lithotrophs or chemoautotrophs. Most of these organisms obtain all their carbon from CO_2 and thus are true autotrophs (Microbial Physiology, Subtopic 2.211). Microorganisms oxidizing organic compounds are called organotrophs or chemoheterotrophs. These organisms generally use the carbon from their energy source as a source of cell carbon so are heterotrophic as well as chemotrophic (Microbial Physiology, Subtopic 2.212).	Some examples of oxidations of inorganic materials are the following: $H_2S \rightarrow SO_4^{2-}$ + energy $NH_4^+ \rightarrow NO_2^-$ + energy $H_2 \rightarrow H_2O$ + energy Organisms that carry out these reactions are important in the cycling of elements in the biosphere. Some examples of oxidation of organic materials are the following: glucose ($C_6H_{12}O_6$) $\rightarrow 6CO_2$ + energy stearate ($C_{18}H_{36}O_2$) $\rightarrow 18CO_2$ + energy Generally, the less oxygen an organic molecule contains, the more energy it will release when oxidized to completion.	
2.12	Phototrophs	Microbial phototrophs are a class of photosynthetic organisms which obtain energy from light.	Some examples are eucaryotic algae and procaryotic blue-green algae (cyanobacteria), photosynthetic bacteria. Eucaryotic algae carry out photosynthesis by using chlorophyll packaged into chloroplasts and evolve O_2. Cyanobacteria have chlorophyll and evolve O_2. The green and purple bacterial phototrophs do not have chloroplasts but do have specialized membrane structures containing bacteriochlorophyll; no O_2 is formed.	The Winogradsky column is an effective way to demonstrate a variety of microbial phototrophs. These bacteria grow under anaerobic conditions in the light, and populations having different pigments develop in different microhabitats in the column. Development of a column takes about four weeks (*Carolina Tips*, September 1978. Vol. XVI, #9).
2.2	Required Elements and Compounds	The elements C, H, N, O, P, and S make up 99% of the dry cell mass and must there-		To determine a requirement of a particular microorganism, the growth yield is com-

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		fore be supplied in greatest amounts. Depending upon the synthetic capacity of the particular microorganism, these elements may be supplied as organic or inorganic materials and in their oxidized form or reduced form.		pared on media with and without the compound.
2.21	Carbon	Carbon is required for the "skeleton" of all of the organic molecules.	All naturally occurring carbon compounds can be used as a source of carbon and energy by some microorganism. There are man-made organic compounds (plastics, DDT) which are decomposed slowly, if at all, by microorganisms. Some bacteria can use more than 90 different compounds, but others are limited to a few. For example, members of the genus *Pseudomonas* are noted for their versatility, whereas organisms such as *Leptospira* are very restricted in their carbon source utilization.	You can demonstrate organisms which utilize a particular carbon source by setting up an enrichment culture. This involves inoculating 100 ml of a mineral medium (such as the one in Subtopic 2.211 adjusted to pH 7.0) with 1 g of soil or several milliliters of pond water and adding an appropriate amount of the carbon source of your choice (0.05 to 1.0%, depending on toxicity). After 1 week or so transfer about 1 ml of this culture to a new flask made up the same way. A large proportion of the organisms growing in the second flask will be able to utilize the added carbon source.
2.211	Autotrophs	The autotrophs can obtain all of their carbon requirements from CO_2. Photoautotrophs are those which use light as an energy source and CO_2 as a carbon source. Chemoautotrophs use chemical sources of energy and CO_2 as the source of carbon. These organisms oxidize inorganic materials as a source of energy and can also be called lithoautotrophs.	Some examples of photoautotrophs are plants, eucaryotic algae, blue-green bacteria (cyanobacteria), and certain photosynthetic bacteria. Some examples of chemoautotrophs are *Nitrobacter*, *Thiobacillus*, and the hydrogen-oxidizing bacteria. *Nitrobacter* oxidizes nitrogen ($NO_2^- \rightarrow NO_3^-$), and *Thiobacillus* oxidizes sulfur ($H_2S \rightarrow SO_4^{-2}$).	This medium will support autotrophic growth of sulfur-oxidizing bacteria. The carbon comes from the CO_2 in the atmosphere, and the energy comes from added sulfur or thiosulfate. Add powdered sulfur to 1%. $(NH_4)_2SO_4$ 0.4 g KH_2PO_4 4.0 g $CaCl_2$ 0.25 g $MgSO_4 \cdot 7H_2O$ 0.5 g $FeSO_4$ 0.01 g Water 1,000 ml
2.212	Heterotrophs	The heterotrophs obtain their carbon requirement from organic molecules.	Photoheterotrophs, e.g., some purple bacteria, use light as their energy source and organic carbon as their carbon source. Chemoheterotrophs, e.g., fungi, most bacteria, use organic carbon as both carbon source and energy source.	In addition to an organic compound, supplemental CO_2 may be required by heterotrophs, e.g., *Neisseria*. A candle jar provides adequate amounts of CO_2 for these organisms.
2.22	Nitrogen	Nitrogen is found predominately in proteins and nucleic acids. It is available in	Many microorganisms obtain organic nitrogen by excreting proteolytic enzymes to	Free-living nitrogen-fixing bacteria may be obtained by inoculating nitrogen-free me-

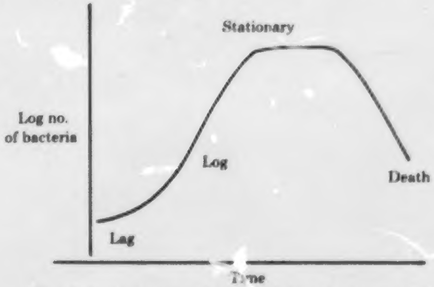
TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		many forms. Atmospheric N_2 may be reduced and assimilated by nitrogen-fixing organisms such as *Azotobacter*. Other organisms can utilize NO_3^- or NH_4^+. Organic nitrogen sources such as amino acids may be required by others.	decompose proteins. They then take the released amino acids into their cells. Only procaryotic organisms seem to be able to syntheaize the enzymes necessary for nitrogen fixation. **Brill, W. J.** 1977. Biological nitrogen fixation. Sci. Am. **236**:68–82.	dium with a small amount of soil and incubating this culture in the dark for 48 h. *Azotobacter* is commonly isolated by this technique. *Rhizobium*, a nitrogen-fixing symbiont, can be observed by crushing the nodules on the roots of legumes and staining the cells released.
2.23	Phosphorus	Cell phosphorous is found predominately in nucleic acids, nucleotides, and phospholipids. Phosphorous is usually added to growth media as an inorganic salt.	Phosphate is actively transported by membrane-bound carriers. In most aquatic systems, phosphate is a limiting nutrient. Addition of phosphate, e.g., phosphate contained in cleaning compounds, to streams may result in increased growth of microorganisms.	In growth media phosphorous is usually supplied at 0.01 to 0.1 M by a buffering mixture of KH_2PO_4–K_2HPO_4.
2.24	Other Elements	Sulfur, found predominantly in proteins, may be obtained from organic sources such as cysteine or inorganic sources such as SO_4^{-2}. Oxygen for nutritional purposes is provided mainly by oxygen-containing compounds. Cations such as K^+, Mg^{2+}, Ca^{2+}, and Fe^{2+} are required by most cells and usually must be added to the medium. Cations such as Mn^{2+}, Co^{2+}, Cu^{2+}, Mo^{2+}, and Zn^{2+} are considered as trace elements.	Bulk cations are provided as salts. K^+ is required for protein synthesis. Mg^{2+} is used as a cofactor for enzymes and functions in maintaining the structure of ribosomes. Fe^{2+} is required for synthesis of cytochromes and other enzymes and coenzymes. Growth of some pathogens in vivo may be limited by the ability of host iron-binding factors to render this element unavailable to the bacteria. Ca^{2+} contributes to heat resistance of endospores. The function of the trace elements is difficult to establish. Most are associated with specific enzyme activities, but Co^{2+} is a component of vitamin B_{12} and Mo^{2+} is required for nitrogen fixation and NO_3^- reduction.	Trace elements may usually be supplied in adequate amounts by tap water or as contaminants in various media constituents. There is sufficient Mo^{2+} in most Mg_2SO_4 to supply trace needs.
2.25	Other Compounds	Water makes up 75 to 90% of the weight of most cells and must be provided. Growth factors are those precursors to cell constituents that specific microorganisms are unable to synthesize from simple materials; they must be provided preformed. These are organic molecules required in small amounts.	Water in humid air is available to free-living fungi and bacteria. In the tropics, for example, care must be taken to inhibit growth on materials normally thought to be too dry to support growth, such as leather and glass. Vitamins, as defined by human requirements, are also required for microbial metabolism. Many microorganisms can synthesize vitamins and thus they may not be required in the medium. Vitamins, amino	The amount of available water is expressed as water activity (a_w). You can preserve food by decreasing available water either by drying or by adding water-binding compounds such as sugar or salt. Fungi can grow at lower water activities than most other microorganisms.

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		acids, and other growth factors may be required by some species. If growth factors are required, the amount of growth that results is directly proportional to the amount of growth factor added to the medium. This is the basis for many quantitative bioassays. Some bacteria, yeasts, and filamentous fungi will synthesize excess vitamins. These microorganisms are used for commercial production of these substances.	
2.3 Media	Media are composed of compounds that are required for growth of microorganisms. No one medium will support the requirements of all microorganisms. Isolation and growth of any organism are dependent on the use of an appropriate medium.	A general purpose medium provides conditions that will support growth of a variety of microorganisms, e.g., Trypticase soy agar (BBL Microbiological Systems). An enriched medium is one to which special nutrients have been added to enhance the growth of a specific microorganism, e.g., blood agar. A differential medium contains components which permit presumptive identification of certain classes of microorganisms based on their biological differences, e.g., lactose-fermenting colonies turn pink on McConkey agar. Selective media are media which preferentially permit the growth of a certain desired microorganism. Selection may be achieved by addition of inhibitors or by adjusting nutrient content to optimize growth of the desired organism, e.g., mannitol-salt agar selectively permits growth of staphylococci. The use of media to enhance growth of specific physiological types of microorganisms is commonly called "enrichment culture." Defined or synthetic media are those in which all the components are known in kind and amount. Complex media are those which contain plant or animal extracts of variable composition.	Agar, used most frequently as a solidifying agent at 1.5 to 2.0% for media, is a polysaccharide obtained from seaweed. It is not metabolized by most bacteria. Aqueous 2.0% suspensions melt at 100°C and gel at 45°C. Silica gel which has no organic impurities may be used as a solidifying agent when agar is either toxic or metabolized. Extracts or enzymic digests of plant or animal tissue provide a rich supply of nutrients. Examples are tryptone, peptone, and yeast extracts. Most media sold commercially are complex; however, there are defined or synthetic media also available.

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
3.0	**Growth**	Growth is the orderly increase in cellular constituents. Normal growth leads to cell reproduction. Growth of microorganisms leads to an increase in the population.	In organisms that reproduce by binary fission, e.g., most bacteria, growth yields increased numbers. The organisms that are coenocytic increase the size of the individual rather than its numbers. Disorders of metabolism and growth can lead to situations in which the normal growth and reproduction cycle is aborted and unbalanced growth results.	
3.1	Measurement of Growth	It is possible to measure various aspects of microbial growth. One chooses the growth measurement technique needed depending on whether the emphasis is on total numbers, viable organisms, or population metabolic rate. Usually several techniques are used, and the results are compared.		
3.11	Increase in Mass	Measurements of increase in cell mass emphasize growth rather than reproduction. They may not distinguish living from dead cells.	Nephelometry and spectrophotometry are the most convenient and generally used growth-measuring techniques for bacteria. The increase in turbidity of the culture is measured. Spectrophotometers measure the proportion of light which is transmitted through a filled cuvette placed in the light beam. Nephelometers (more sensitive) measure the proportion of light deflected under the same conditions.	A sample of washed cells may be dried in the oven, and dry weight can be determined. A sample of washed cells may be analyzed to determine the total nitrogen or total protein. Cells in suspension scatter light; the amount of light scattered is proportional to the mass of cells per milliliter of fluid. A spectrophotometer or nephelometer can be used to measure scattered light.
3.12	Increase in Numbers	Cell numbers can be measured by direct counting with a microscope. Culture techniques which test the ability of each organism present to grow to a visible mass or to metabolize and produce a detectable product are also used (viable counts). These methods are useful only for unicellular organisms.	Direct counts may be done to assist in standardizing other methods for routine use. They are also useful for quantitating unusual organisms in natural samples or hard-to-cultivate species. Automated electronic particle counters may be used. Viable counts are much used in studies of water samples, urinalysis, and food quality determination. A diluted sample is spread on a solid or mixed into a melted agar medium. If this is done with medium in a petri plate, it is called a plate count. Each viable organism may grow into a visible mass of	The direct observation of a stained smear of a known volume of a specimen spread over a known area on slide can be used to calculate numbers of organisms per milliliter. The Petroff-Hauser counting chamber is used for direct counts of bacteria and other small cellular organisms, and the count reflects both living and dead microorganisms. Use the plate count to determine the number of CFU in a sample under several nutrient and physical conditions.

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			cells (a colony). The number of colonies after incubation of this medium represents the number of viable bacteria in the diluted sample. Because it is difficult to know if one bacterium or several attached bacteria gave rise to one colony the number is usually given as colony-forming units (CFU).	
3.2	Factors Influencing Growth	Microorganisms are heterogeneous in their physical and chemical requirements. Optimum growth occurs only when all requirements are met.	When microorganisms are not growing because of inadequate conditions, they are not necessarily dead. Long periods of metabolic inactivity may be tolerated without loss of viability.	
3.21	Nutrition	Growth patterns differ depending on whether nutrient supply is continuous (open system) or discontinuous (closed system). Most natural situations are open; most laboratory conditions are closed.	(Microbial Physiology, Subtopics 3.3 and 3.4)	
3.211	Open Versus Closed Systems of Microbial Growth	Microorganisms contain regulatory mechanisms which allow them to modulate growth for survival under changing nutrient availability.	In natural situations such as bodies of water, organisms receive a continuous if fluctuating supply of nutrients. Wastes are also removed. Growth speeds up and slows down, rarely stops. In the test tube, when the nutrient is consumed, growth stops.	
3.22	Physical and Chemical Influences on Growth			
3.221	Gaseous Atmosphere	Microbial growth is affected by the concentrations of O_2, CO_2, and other gases in the surrounding medium. Most higher forms of life require O_2; many microorganisms do not. Obligate aerobes require O_2 for growth. Their metabolism is respiratory. Facultative anaerobes grow either with O_2 or without it. Growth is usually better in the presence of O_2. These organisms are usually fermentative in the absence of O_2. Obligate anaerobes do not grow in the presence of oxygen. Microaerophiles require reduced O_2 concentration and may require increased CO_2 concen-	Obligate anaerobes may be recovered from conditions in nature which appear to be aerobic. These organisms grow in microenvironments that are anaerobic. *Bacteroides*, an obligate anaerobe, can be isolated from the mouth. It inhabits anaerobic crevices around the teeth. Many anaerobes are killed by oxygen. Isolation from natural habitats (anaerobic mud and soil, the guts of animals, wounds, and absesses) requires special sampling techniques, transport conditions, and prereduced culture media.	Aerobes and facultative anaerobes must have an O_2 source for best growth. In liquid culture best yields are obtained with small volumes of fluid in large vessels on shakers. Anaerobic growth conditions can be provided in sealed jars with catalytic oxygen removal or in anaerobic incubators. Thioglycollate, chopped meat medium, and media covered with mineral oil may be used to provide anaerobic conditions. High-CO_2, low-O_2 atmosphere can be provided in a candle jar by combustion or in specially designed incubators.

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	tration. Aerotolerant anaerobes are organisms living totally by fermentation in the presence of air. They are not sensitive to O_2.		The catalase and oxidase tests may be useful in identification of bacteria. **E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.).** 1980. Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
3.222 pH	For most organisms the optimum pH for growth lies between 6.0 and 7.5. Some highly specialized organisms can tolerate a pH as low as 1.0 or as high as 10.4. The internal pH of most microorganisms is close to neutrality regardless of the pH of the medium. Microbial metabolism produces acidic or basic waste products. These may alter the environmental pH to a point where growth stops. Laboratory media are usually buffered to reduce this effect.	Extreme pH occurs in some natural environments. Drainage from mining operations, high mineral areas, and certain fermentation conditions yield high-acidic environments. The inland salt or alkali lakes may have a pH as high as 11. *Thiobacillus* is an autotroph which produces H_2SO_4 and lowers pH of medium to 1.0 or below before growth stops. Fermentative organisms such as those used to make yogurt or sauerkraut lower pH by production of organic acids. These acids eventually arrest growth. They preserve food by preventing growth of less acid-tolerant organisms that would spoil foods. Many important biochemical tests used in identification of bacteria depend on pH indicators to detect production of acidic or basic end products.	The complex peptones, cell extracts, and other protein derivatives in most complex media exert a strong buffering effect. Note pH information on media labels. A variety of inorganic buffers may be added to defined media. Detection of pH change in media is usually done with pH indicators, e.g., phenol red is yellow below pH 6.8, and red above 6.8; methyl red is red below pH 4.5 and yellow above 4.5. Electronic pH meters can be used for exact readings in liquid media. Selective media for the isolation of fungi usually have a pH below 6.0, whereas most general-purpose bacteriological media have a pH of 6.8 to 7.4.
3.223 Temperature	A given strain of microorganism will have an optimum temperature for growth. Growth will occur between its minimum temperature and its maximum temperature. These points are genetically determined. The effect of temperature on enzyme activity (Microbial Physiology, Subtopic 4.53) is important in determining the range over which an organism will grow. There are three groups of microorganisms with different ranges of growth temperatures: psychrophiles, mesophiles, and thermophiles. One commonly used definition of psychrophiles is that they have a growth range somewhere between −5 and 20°C. Meso-	Microorganisms grow over their entire growth temperature range. At suboptimal temperatures their generation time is extended from minutes to hours. Above the optimum, growth slows as the microorganism diverts ever-increasing amounts of cellular energy to repair heat-induced damage. The upper cut-off point is usually quite sharp. Thermophilic procaryotes can withstand higher temperatures than eucaryotic thermophiles. The hottest inhabited thermal springs contain only procaryotes. *Thermus aquaticus*, a thermophilic bacterium, is found growing in hot water heaters as well as in hot springs.	Soil or water samples may be plated and incubated at different temperatures to demonstrate presence of all three types of organisms. Many psychrophiles are killed by exposure to room temperature, so precautions must be taken to keep samples and media cold.

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	philes grow between 20 and 45°C. Thermophiles grow between 45 and 90°C.		
3.224 Osmotic Pressure	Osmotic pressure is determined by the concentration of dissolved particles in water. Microorganisms are found in environments of varying osmotic pressures. Most microorganisms with the exception of protozoans are protected from lysis in dilute environments by a rigid cell wall. Exposure to high osmotic pressure causes loss of water from cells. This arrests growth. The halophilic organisms live in highly saline environments (15 to 30% dissolved salts). They have a specialized membrane and salt-tolerant enzymes.	All types of dissolved and colloidal particles contribute to the osmotic pressure of a solution. Cytoplasm has a relatively high osmotic pressure. The cell can vary this within limits by concentrating or excreting K^+. When the external environment contains more dissolved material than the cytoplasm, it is hypertonic, and the cell will tend to lose water to the environment. This drying effect arrests metabolism in most organisms; the effect explains the preservative value of salting or sugaring food. Dilute environments pose a challenge to bacterial survival. When the exterior environment is hypotonic, water tends to enter the cell. Expansion and death are prevented by the rigid cell wall. Cell wall-deficient forms of bacteria survive only in isotonic environments. Examples are the L-forms and mycoplasms.	Mannitol salt agar may be used for the selective isolation of *Staphylococcus* from human clinical samples because its high (7.5%) NaCl concentration inhibits almost all other nasopharyngeal organisms. The osmotic pressure of media for isolation of cell wall-deficient organisms, e.g., *Mycoplasm*, must be carefully adjusted.
3.3 Closed System Growth	Within a closed system the amounts of nutrients and their energy content are fixed. Growth produces waste products which accumulate and may be toxic. Space for growth or attachment to substrate becomes limiting.	 FIG. 1. *Phases of microbial growth in closed culture.*	All laboratory cultivation of microorganisms except continuous culture follows this model. The phases of growth may be demonstrated by inoculating fresh, prewarmed medium with an inoculum of log-phase cells and following the increase in medium turbidity. Any other accurate measurement of cell components may be used. By taking simultaneous samples for plate count and direct count, a good approximation of actual numbers may be obtained for each reading.
3.31 Lag Phase	Introduction of a microorganism that divides by binary fission into a sterile closed system such as a flask of nutrient broth starts growth. For a while there is no appreciable increase in numbers.	Each cell in the innoculum needs to carry out internal regulatory shifts to start growth. Accumulation of cytoplasmic components and increase in cell size begin. During lag phase, new proteins may need to be made. A complete round of DNA replication will precede the first and every subsequent cell division.	The duration of lag phase is extended if the inoculum is from an old culture. If the new medium is quite different in chemical composition from the old, or if it is at refrigerator temperature. During lag phase, measurement of cell numbers shows no change. Some cell constituents may increase.

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
3.32	Log Phase (Exponential Growth)	Once growth begins, increase in numbers is exponential for a period of time. The culture will commence division at the maximum rate possible given the genetic potential of the culture and the suitability of the medium.	During log-phase growth, readings should be made frequently because turbidity can double in as little as 20 to 30 min for some bacteria. Growth curve data may be plotted on linear graph paper with time as the abscissa and the log of the turbidity or the log of the number of organisms as the ordinate. It is more convenient to use semilog paper; the cell number or turbidity is plotted on the log axis, and the time is plotted on the linear axis. Both of these plotting methods will produce a straight line if the culture is in exponential growth (Fig. 2).	
3.321	Explanation of Exponential Growth	For most microorganisms one cell divides into two. Thus, during each generation time the population doubles. Starting with one bacterium, you can illustrate exponential growth with the geometric progression: 1, 2, 4, 8, 16, 32 … or $2^0, 2^1, 2^2, 2^3, 2^4, 2^5 \ldots 2^n$.	All populations grow or decrease exponentially if each change reflects addition or subtraction of some constant percentage of the total individuals present at that time. If exponential growth proceeded for 48 h, one bacterium which divided every 20 min would yield a quantity of bacteria weighing 4,000 times the weight of the earth.	During exponential growth there is a straight-line relationship between the log of cell number versus time. When data are plotted, the generation time can be estimated by determining the time needed for a doubling of numbers or cell mass. Specific data points may be taken from the straight-line portion of the plot to use in a more precise calculation of generation time.
3.322	Generation Time	The generation time observed is the average time needed for one cell to complete a round of division into two cells. In this time the population in the closed system will double. The total amount of each cell constituent will double.	Generation times for some bacteria may be as short as 12 min. Thus, bacteria can multiply extremely rapidly under optimum conditions. Generation times for other bacteria and many eucaryotic microorganisms may be as long as several days.	Calculate generation time as follows: $G = \dfrac{t_1 - t_0}{n}$ or $\dfrac{t_1 - t_0}{(3.3)\log_{10}b_1 - \log_{10}b_0}$ G = doubling time or generation time t_0 = time at first measurement t_1 = time at second measurement b_0 = number of cells at t_0 b_1 = number of cells at t_1 n = number of generations
3.33	Stationary Phase	Environmental limitations put an end to exponential growth. The growth curve approaches a horizontal line. During the stationary phase, some cells grow and divide.	Nutrient limitation is the major restriction for most aerobic organisms. Accumulation of toxic wastes is the most frequent cause of growth arrest in anaerobic culture.	The stationary phase may persist for a long period. Readings should be less frequent.

Data

Time	No. of organisms
0	1×10^7/ml
20	2×10^7/ml
40	4×10^7/ml
60	8×10^7/ml

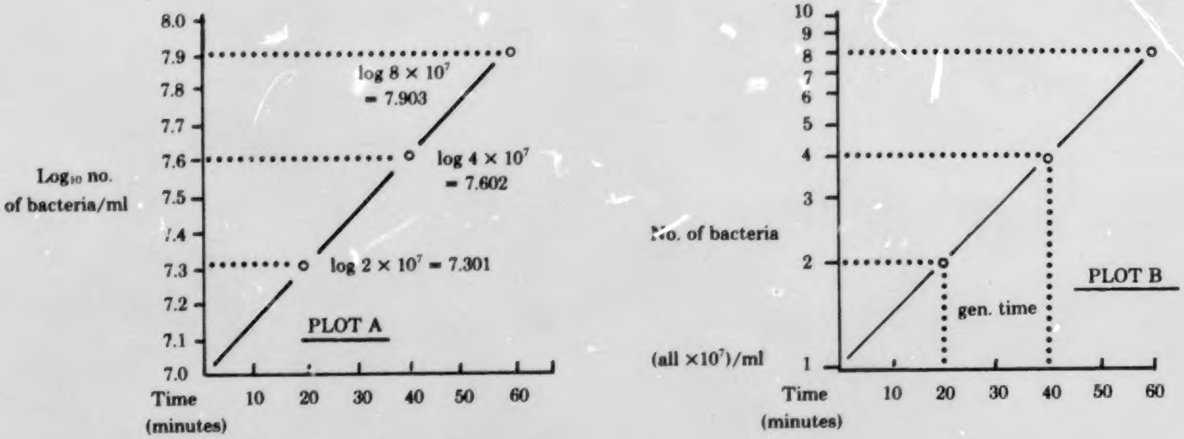


Fig. 2. *Plots of exponential growth.*

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		Some are active, others die, and the population number is unchanged.		
3.34	Death Phase	A decline in number of viable cells constitutes death phase.	Death phase results in a reduction in viable cell numbers. It may or may not have a corresponding decline in total cell numbers.	The decline is usually exponential. Small numbers of viable cells may persist indefinitely at the end of the death phase.
3.341	Mechanism	Acute nutrient limitation or high concentrations of toxic waste products may trigger activity of autolytic enzymes causing cell lysis.	When cells lose viability without lysing, this loss can usually be attributed to inability to continue supplying the energy to repair key genetic structures. When autolysis occurs, it is usually related to low cellular adenosine triphosphate (ATP) levels, signaling activation of intracellular lysozyme. Clearing of the culture may be quite rapid if autolytic mechanisms are set in motion. There may be no viable survivors.	
3.4	Open System Growth	Open system growth occurs when constant environmental conditions are maintained. Nutrients are continuously provided, and wastes are removed. The number of cells per unit volume is kept constant.		
3.41	Conditions	Laboratory devices for producing open system growth provide a source of cells in the log phase of growth. They allow study of a culture under optimum physiological conditions.	The growth rate of the bacteria in the vessel adjusts to the rate at which the nutrients are provided. After a period of adjustment, the rate of increase of the cells through growth will just equal the rate of cell loss. The cells in a continuous culture apparatus are in log phase. The doubling time is determined by the growth conditions. If all else is constant, growth rate depends directly on the concentration of a limiting nutritional factor.	Growth occurs in a growth chamber provided with a reservoir of sterile medium that is added at a steady rate. Culture fluid leaves the chamber at the same time. Aeration and mixing are also provided.
3.42	Continuous Culture Devices	These are two types of continuous culture devices, chemostats and turbidostats. They are used for study of all activities of log-phase cells.	Continuous culture systems provide a convenient constant source of log-phase cells for study. They are used for research on mechanisms of regulation, concentration of nutrients, selection of growth rate mutants, and interactions among species in mixed cultures under conditions which stimulate natural environments.	In a chemostat, the flow rate is set at a certain value. The rate of growth of the culture adjusts to the rate at which nutrients are added. In a turbidostat, an electronic device monitors turbidity, i.e., cell mass, of the fluid and electronically signals the addition of fresh medium to maintain the desired population density.

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
4.0	**Enzymes**			
4.1	Definitions			
4.11	Catalyst	A catalyst is a substance which affects the rate of a chemical reaction without being permanently altered itself.	Catalysts are used in a number of reactions of everyday practical interest and industrial importance. For example, platinum catalytically enhances the oxidation of unburned hydrocarbons in automotive exhaust (catalytic converters). Also, the industrial extraction of apple juice from apple pulp is aided by treatment with pectin-hydrolyzing enzymes (pectinases).	Starch + Water → Glucose The above reaction will proceed at a very low rate in warm water. Upon addition of a small quantity of amylase, accelerated glucose production (and starch hydrolysis) is noted. After a time, the enzyme can be isolated from the system with its original activity essentially undiminished.
4.12	Enzymes	An enzyme is a highly specific catalyst produced by a living cell. Enzymes have a protein component. They may or may not have other components such as metal ions, vitamin, or carbohydrate molecules.	The specificity of enzymes extends even to the ability to "recognize" specific isomeric forms of a potential substrate. For example, a particular lactate dehydrogenase may discriminate between D-lactic acid and L-lactic acid. The only structural difference is the relative position of "H" and "OH" groups on the second carbon of this three-carbon compound.	The fact that enzymes are protein can be demonstrated by their reaction with protein-detecting reagents such as Millon reagent or the Folin phenol reagent. Enzyme solutions will also exhibit maximum ultraviolet absorbance at 280 nm, which is characteristic of protein solutions.
4.13	Substrate	A substrate is a substance which is altered in an enzyme-catalyzed reaction.		
4.2	Structure	Some enzymes consist only of protein.		
4.21	Holoenzyme	A holoenzyme is the functional form of an enzyme. Some holoenzymes consist of apoenzymes, coenzyme, or an inorganic factor.		
4.22	Apoenzymes	The apoenzyme is the specific protein portion.		
4.23	Coenzymes	Coenzymes are vitamin derivatives which serve as carriers in enzyme reactions.	Coenzymes will often act as donors or acceptors of electron pairs or functional groups. In the oxidation of malic acid to oxaloacetic acid in the tricarboxylic acid cycle, nicotinamide adenine dinucleotide (NAD) which is derived from the vitamin niacin, acts as an electron and proton acceptor. Pyridoxamine phosphate (derived from	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION
			the vitamin pyridoxal phosphate) acts as an amino group donor in transamination reactions. Other commonly encountered coenzymes include: flavine adenine dinucleotide (FAD, derived from riboflavin); nicotinamide adenine dinucleotide phosphate (NADP, derived from niacin); coenzyme A (CoA, derived from pantothenic acid); and thiamine pyrophosphate (TPP, derived from thiamine). Many microorganisms can synthesize all of these cofactors.
4.24	Inorganic Cofactors	An inorganic cofactor is a metal ion, such as Mg^{2+} or Fe^{2+}, which is required by a specific enzyme for catalytic activity.	In some enzymes the metal ion may serve as an important part of the catalytic site. In other reactions the metal ion is involved in substrate binding or in maintaining the active conformational form of the enzyme.
4.25	Active Site	An active site is a small area on the enzyme's surface which binds in a highly specific manner to the substrate. It is also called the catalytic site.	The active site can be pictured as a structure which matches up with a specific complementary conformation in the substrate in a "lock and key" sort of arrangement (Fig. 3).
4.26	Allosteric Site	An allosteric site is a region on the enzyme surface, apart from the active site, where a regulatory substance may bind and affect the affinity of the enzyme for the substrate.	The allosteric site can be pictured as shown in Fig. 4. Note that the active site will "fit" the substrate but not the allosteric effector. The effector, if bound, results in a modification of the three-dimensional structure of the apoenzyme. This, in turn, modifies the structure of the active site.

C. PRACTICAL ACTIVITIES

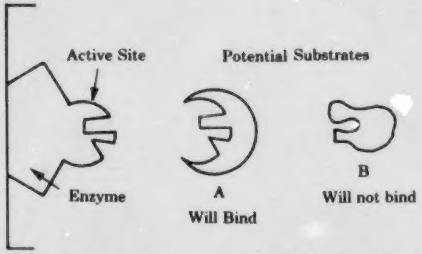
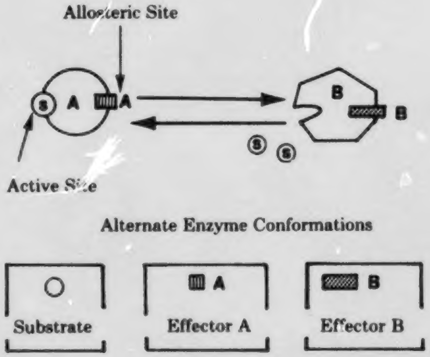


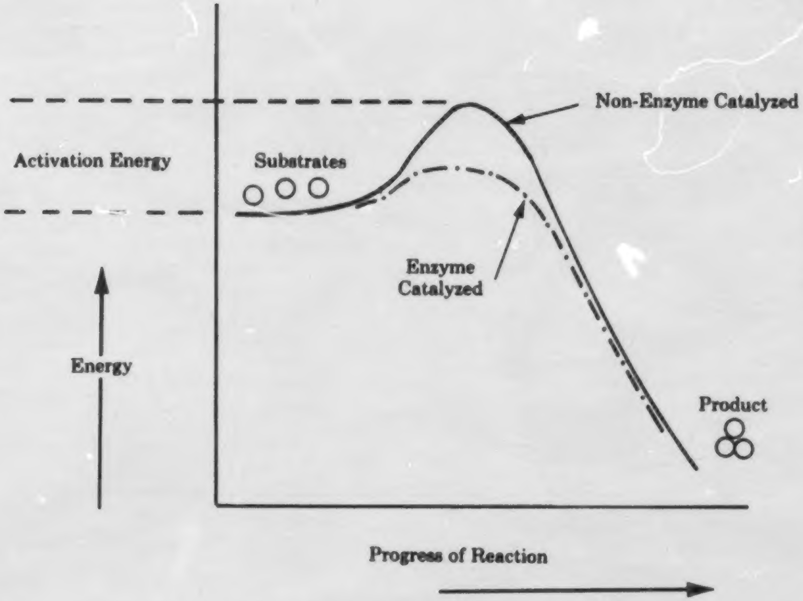
FIG. 3. *Complementary structure of active site and substrate*



If effector A is present, enzyme will be in a favorable conformation for interaction with the substrate. If effector B is present, substrate binding will be reduced because this effector stabilizes conformation B which cannot bind substrate.

FIG. 4. *Allosteric site and binding of positive and negative effectors.*

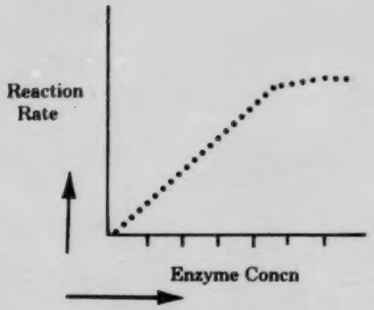
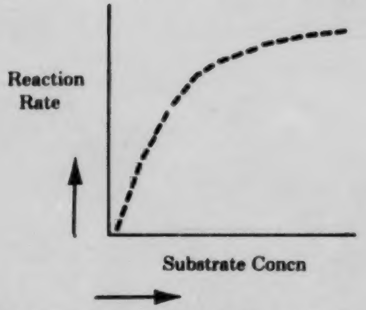
TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
4.3 Mechanisms			
4.31 Activation Energy	The minimum energy required by a chemical system in order to react is referred to as the activation energy. Enzymes work by lowering the required activation energy, thus facilitating the reaction (Fig. 5).		
4.32 Enzyme-Substrate Complex	A substrate (S) binds temporarily to the active site of an enzyme (E) to form an enzyme-substrate complex (ES). The ES dissociates to yield product (P) and enzyme (E), which is free to catalyze another reaction. $E + S \rightleftarrows ES \rightleftarrows E + P$		
4.33 Equilibrium	Under optimal conditions the concentrations of enzyme, substrate, and product in a system reach equilibrium. This involves the simultaneous conversion of some molecules of substrate into product and product into substrate.	Once equilibrium is established, constant concentrations of substrate and enzyme are present in the system unless the equilibrium is disrupted in some manner. In fact, some substrate is continuously being converted to product and some product is converted to substrate. Thus, although the concentrations remain constant, the individual molecules are in a continuous state of interaction. In biological systems, utilization or removal of products can keep reactions from coming to equilibrium.	Equilibrium can be demonstrated with a hypothetical system consisting of radioactively labeled substrate and unlabeled product. $A^* \rightleftarrows B$ Initially, all the label is in the substrate. Eventually however, the following will be observed: $A^* \rightleftarrows B^*$ In this second instance, the label is distributed between the substrate "A" and the product "B". Yet, the actual concentrations of A and B could be the same as in the initial example.
4.4 Nomenclature	Enzymes are usually named according to the type of reaction catalyzed; they often have the common suffix -ase.	Enzymes have common names established by long usage as well as official names and numbers designated by the Enzyme Commission. For example, invertase, the enzyme that hydrolyses sucrose to fructose and glucose is officially called β-D-fructofuranoside fructohydrolase (EC 3.2.1.26).	

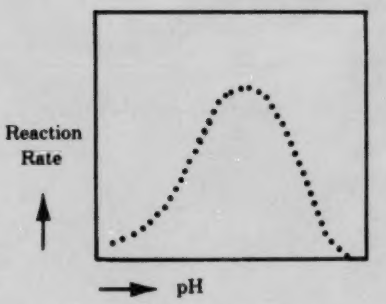
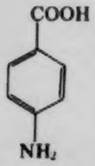
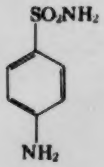

Non-Enzyme Catalyzed
Activation Energy
Substrates
Enzyme Catalyzed
Energy
Product
Progress of Reaction

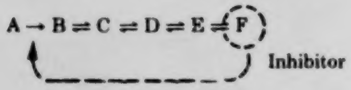
Fɪɢ. 5. *Enzymes and activation energy of reactions.*

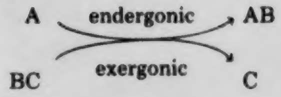
Substrate molecules are visualized as balls near the top of a hill. If they could roll down the hill, they would have tremendous kinetic energy. However, they are trapped in an "energy depression." To escape this requires a "push" of activation energy.

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
4.41 Oxidoreductases	Enzymes of this type catalyze the intermolecular transfer of electrons.	Generally electrons are transferred with protons. NAD^+ and FAD are common proton acceptors in oxidoreductase reactions and, in their reduced form (NADH, $FADH_2$), are proton donors. An example of an oxidoreductase reaction is the interconversion of lactate and pyruvate by lactate dehydrogenase.	
4.42 Transferases	Enzymes of this type catalyze the intermolecular transfer of functional groups.	The conversion of α-ketoglutarate to glutamic acid is catalyzed by a transaminase which moves an amino group from aspartate to α-ketoglutarate.	
4.43 Hydrolases	Enzymes of this type catalyze hydrolytic reactions.	A hydrolytic reaction is the breaking of a covalent bond by the addition of one molecule of water. Preliminary digestion of food molecules is accomplished by exoenzymes of this type.	
4.44 Lyases	Some enzymes of this type catalyze the addition of groups of atoms across a double bond. Other enzymes of this group catalyze cleavage of C—C, C—O, or C—N bonds by elimination reactions leaving double bonds.	An example of the first type of lyase reaction is the addition of water across the double bond of fumarate to form malate. This is catalyzed by fumarase one of the tricarboxyclic acid cycle enzymes. Aldolase is an example of lyase able to cleave C—C bonds.	
4.45 Isomerases	Enzymes of this type catalyze the interconversion of isomers.		This is demonstrable with two cultures, one of which is genetically able to produce galactose isomerase, whereas the other is not. In a medium containing galactose as the sole carbohydrate, the organism which produces the enzyme will exhibit evidence of galactose utilization (such as gas production in a fermentation tube), whereas the other organism will not. Such reactions are of significance in the identification of unknown microorganisms.
4.46 Ligases	Enzymes of this type catalyze energy-requiring reactions involved in biosynthesis.	Assembly of macromolecules from monomers typifies ligase action. Nonphotosynthetic CO_2 fixation catalyzed by pyruvate carboxylase is another example. CO_2 + Pyruvate + Energy → Oxaloacetate	

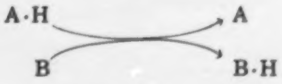
	TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
4.5	Methods of Measurement	Rates of reactions may be measured in terms of disappearance of substrate or appearance of product with time.	Enzyme activity is commonly expressed either as a "turnover number," the number of molecules of substrate converted to product per unit time, or as specific activity, which is the number of molecules of substrate converted to product per unit time per milligram of protein.	A variety of techniques are possible: Spectrophotometry: Frequently, it is possible to observe enzyme activity in terms of the production, with time, of materials with characteristic absorption spectra. For example, polygalacturonic acid lyase, an enzyme involved in the degradation of pectin, produces a product which absorbs maximally at 235 nm. Photometry: Firefly extract (luciferin-luciferase) emits light in the presence of ATP. The rate of ATP utilization in a ligase reaction can be quantitated by measuring the light emitted by an enzyme-substrate system at various intervals. Product generation or substrate utilization can also be detected by a variety of chromatographic and radioisotope techniques.
4.6	Factors Affecting Rate of Enzyme Reactions	The most important factors affecting the rate of enzyme reactions are: enzyme concentration, substrate concentration, temperature, and pH.	 Fɪɢ. 6. *Enzyme concentration and reaction rate.*	
4.61	Enzyme Concentration	Within limits, a linear relationship exists between enzyme concentration and rate of reaction (Fig. 6). Eventually, however, all available substrate molecules are bound to enzymes; thus, an additional increase in enzyme concentration will not cause an additional increase in the reaction rate.		
4.62	Substrate Concentration	As with enzyme concentration, a linear relationship exists between substrate concentration and the rate of reaction (Fig. 7). Similarly, a point is reached where all active sites are bound to substrate molecules. Further additions of substrate beyond this point do not further increase the rate of reaction.	 Fɪɢ. 7. *Substrate concentration and reaction rate.*	
4.63	Temperature	Each enzyme has a specific optimum temperature. Starting below the optimum, raising the temperature increases the reaction rate until the optimum is reached. Further-	Generally, for each 10°C rise (up to the optimum temperature), a doubling of enzyme activity is observed. At temperatures above the optimum, irreversible modifica-	

	TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		more, heating will lead to thermal inactivation of the enzymes.	tions of the three-dimensional structure of the enzymes (denaturation) will occur, leading to a decrease in enzyme activity.	
4.64	pH	Each enzyme has a specific optimum pH. Deviations (above or below) from this in the system result in reduced rates of reaction (Fig. 8).	The optimal pH for most microbial enzymes is between 6 and 8.	
4.7	Enzyme Inhibition	(Microbial Genetics Subtopic 6.1).		
4.71	Competitive Inhibition	Inhibitors which are structurally similar to the substrate compete with the substrate molecules for enzyme-active sites. As the ratio of inhibitor to substrate is increased, a concomitant decrease in product formation occurs. Competitive inhibition can be reversed by increasing the ratio of substrate to inhibitor.  FIG. 8. *pH and reaction rate.*	Consider the following structures:  (PABA) *para*-aminobenzoic acid  Sulfanilamide Some microorganisms utilize *para*-aminobenzoic acid (PABA) as a substrate in the synthesis of folic acid, a material required for metabolism. The enzyme which normally binds PABA at its active site will also bind sulfanilamide. Therefore, if the ratio of sulfanilamide to PABA is high, the probability of the enzyme reacting with sulfanilamide rather than with its normal substrate is also high. The result is a decrease of folic acid synthesis and suppression of metabolism. Such inhibition is based upon the similarity in structure of normal substrate and inhibitor. Sulfanilamide does not effect the supply of folic acid to mammalian cells because they cannot convert PABA to folic acid and thus require folic acid preformed.	
4.72	Noncompetitive Inhibition	Noncompetitive inhibitors bind at a site on the enzyme other than the active site.	An example of noncompetitive inhibition is the action of heavy metals (Hg^{2+}, Ag^{+}, and	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	Since the active site is not directly affected, increases in the ratio of substrate to inhibitor cannot reverse the inhibition.	others) on the sulfhydryl groups of enzymes. This may, in part, explain the inhibition of growth of some microorganisms by heavy metals.	
4.721 Feedback Inhibition	Feedback inhibition is a special case of noncompetitive inhibition. When the end product of a metabolic pathway is present in excess, it may inhibit the action of one of the early enzymes in the pathway. This inhibition involves the allosteric site (Microbial Genetics, Subtopic 6.2).	An excess of "F" interacts with the allosteric site of the enzyme catalyzing the conversion of A to B. Since production of B is blocked, C will not be produced.  FIG. 9. *Feedback inhibition.*	
4.8 Enzyme Synthesis	(Microbial Genetics, Subtopic 6.1)		
5.0 Metabolism	Metabolism is the sum of all the chemical reactions in the cell.	In general, microbial metabolism is quite similar to the chemical reactions that occur in higher animals and plants. However, upon examination of individual microorganisms there is a marked diversity of metabolic pathways and end products. This diversity is useful in identifying bacteria and fungi. Many metabolic pathways known to be similar in all organisms were first worked out by investigators with microorganisms. *Escherichia coli* and *Saccharomyces* have been particularly useful in metabolic research.	
5.1 Energy	Energy is the ability to do work.	Microbial movement, growth, and reproduction occur only if there is an energy source. The ultimate source of most biological energy is the sun. A possible exception to this is the deep-sea volcanic oases system where the driving energy is the oxidation of H_2S by bacteria. **Ballard, R. D., and J. F. Grassle.** 1979. Return to oases of the deep. Natl. Geogr. **156**:689–705.	
5.11 Forms of Energy	Energy can exist as light or other kinds of	The form of energy required directly for	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		radiation energy, chemical energy, mechanical energy, or heat.	microbial movement, growth, and reproduction is chemical energy.	
5.12	Transformation of Energy	Energy can be transformed from one form to another.	Not only are forms of energy interconvertible, but different forms of matter are convertible to energy. Oxidation of organic molecules is often said to yield energy. This does not mean that these reactions make energy, but simply result in energy transformation.	
5.121	First Law of Thermodynamics	The total energy and matter in the universe are constant.		
5.122	Second Law of Thermodynamics	During energy transformations, a loss in usable energy occurs as heat is produced, or the system becomes less organized, or both. When systems become less organized they can do less work. This tendency toward disorder is called an increase in entrophy.	All biological processes are inherently inefficient and thus heat producing.	Heat produced during the chemical reactions of microbial metabolism can be measured. A demonstration of this is the increase in internal temperature in compost heaps.
5.13	Chemical Bond Energy	The energy of a molecule can roughly be represented as the sum of the forces holding the atoms together.		If you burn a carbon-containing substance (totally oxidize it to CO_2), the heat produced is a measure of the potentially useful chemical energy of that substance.
5.14	Endergonic and Exergonic Reactions	Making certain bonds between atoms requires energy input (endergonic reaction), but this energy can later be released by breaking the bond (exergonic reaction).	A typical endergonic reaction may be represented by the following. $A + B + \text{energy} \rightarrow AB$ A typical exergonic reaction may be represented as follows. $BC \rightarrow B + C + \text{energy}$	
5.15	Coupled Reactions	Biological transformations of chemical energy can occur because exergonic (energy-yielding) reactions are linked to endergonic (energy-requiring) reactions by common intermediates.	A coupled reaction is the sum of the following. endergonic: $A + B + \text{energy} \rightarrow AB$ exergonic: $BC \rightarrow B + C + \text{energy}$ SUM $A + BC \rightarrow AB + C$ Coupled reactions may also be written as follows. 	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			The reactions will proceed in the direction of the arrows as long as the energy released by the exergonic reaction exceeds that required by the endergonic reaction.	
5.16	Nucleoside Diphosphates and Triphosphates ADP, GDP, UDP, CDP, ATP, GTP, UTP, CTP	These compounds are the most used intermediates between exergonic and endergonic reactions. The conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) for example, provides an efficient short-term energy storage and transfer mechanism in biological systems. The addition of a third phosphate to a nucleoside diphosphate is an endergonic reaction and thus can only occur when this reaction is linked to an exergonic reaction that releases sufficient energy. Enzyme-catalyzed removal of the third phosphate is exergonic; thus, it can be used to drive cellular energy-requiring reactions.	Although removal of the terminal phosphate group from ADP releases about as much energy as the hydrolysis of ATP to ADP (7.3 kcal mol^{-1}, 30.5 kJ mol^{-1}), ADP is not commonly used as an energy intermediate. In some specific reactions, hydrolysis of GTP, UTP, or CTP provides the energy. A conventional symbolism for the "high-energy" bond is ~ so that ATP = AMP ~ P ~ P.	
5.2	Catabolism	Catabolism is the sum of the cellular reactions resulting in conversion of the energy from various organic molecules to energy usable for cellular work. ATP is the most common "useful" cellular energy form.	According to Mitchell's hypothesis (Microbial Physiology, Subtopics 6.1) the gradient of protons across a cell membrane or organelle membrane can be used directly to do cell work. Catabolism can result in production of proton gradients across membranes.	
5.21	Fermentation and Respiration	Fermentation and respiration are the two basic catabolic schemes. In fermentative pathways, the organic molecules being utilized as a source of energy are incompletely oxidized. No external inorganic electron acceptor is necessary (no O_2 is required). ATP is produced only by substrate-level phosphorylation (Microbial Physiology, Subtopic 5.25). The number of ATP molecules produced per molecule of substrate catabolized is small. In respiratory catabolism (sometimes called oxidative catabolism), the organic molecules being utilized are usually oxidized completely to CO_2. The electron transport	It is often important in characterizing bacteria to determine the type of catabolism they carry out. Strict aerobes carry out respiratory catabolism only. Facultative anaerobes can utilize either fermentation or respiration. Aerotolerant anaerobes use fermentative pathways but are not harmed by O_2. Strict anaerobes use fermentative pathways and are harmed by O_2. A few are able to respire by using alternate electron acceptors (Microbial Physiology, Subtopic 5.2432). Two industrial processes using yeast can be used to demonstrate differences between fermentation and respiration. When yeast is	Some microorganisms obtain all their energy by fermentative pathways, some only use respiratory pathways, and some can use either, depending on the presence or absence of oxygen. Simple laboratory tests can be used to determine whether a particular organism is obtaining energy from fermentation or respiration. One such test depends on the ability or inability of an isolate to utilize a particular organic energy source in a sealed tube. Information on oxidation-fermentation (OF) test media can be found below. **Lennette, E. H., A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.)** 1980.

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		system (Microbial Physiology, Subtopic 5.24) serves to transfer electrons to an inorganic electron acceptor. Most of the ATP is produced by oxidative phosphorylation coupled to the electron transport system. The yield of ATP per molecule of growth substrate metabolized is much greater than the yield of ATP in fermentation.	used for alcohol production the vats are kept strictly anerobic. The ethanol is a product of fermentation. When yeast is produced for sale as bakers' yeast it is grown under highly aerobic conditions because the best yield of cells for the substrate added can only be obtained if the yeast is living by respiration.	Manual of Clinical Microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
5.22	Oxidation-Reduction Reactions	Oxidation is removal of electrons and often protons (H^+) from a molecule. The electrons removed must be donated to another molecule, and this recipient is said to be reduced. Thus, an oxidation reaction must always be accompanied by a reduction reaction. Oxidation-reduction reactions result in a transfer of energy. Much of the energy involved in the transfer may be lost to the system as heat, or if the reaction proceeds in a stepwise fashion, some of the energy may be conserved by coupled reactions.	In biological systems, oxygen is one of the most avid available acceptors of electrons (most electronegative). Thus, an oxidation in which oxygen ends up with the transferred electrons releases the most energy.	An oxidation-reduction reaction can be represented as follows.  · = electron H = proton A = electron donor B = electron acceptor.
5.23	Nicotinamide Adenine Dinucleotides as Electron Carriers	Nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) are the most common immediate electron acceptors of organic molecules. Reduced forms of these molecules (NADH and NADPH) act as electron donors in other reactions.	The use of NAD^+ and $NADP^+$ as electron acceptors in catabolic processes is very important to the conservation of energy released by oxidation. NADH and NADPH do not donate electrons to oxygen directly and are relatively stable in the absence of enzymes specific for transfer of the electrons to a given substrate. This enables the organism to maintain control of its oxidation-reduction reactions. NAD^+ is made from niacin and is a good example of the place of vitamins in metabolism.	For directions for performing a lactic dehydrogenase enzyme assay which will demonstrate use of NAD as an electron acceptor consult: **Bergmeyer, H. U.** 1974. Methods of enzymatic analysis, vol. 1. Academic Press, Inc., New York.
5.24	Electron Transport	Electron transport is a cellular mechanism for conserving the energy released during oxidation of organic molecules. Catabolic processes involving participation of the electron transport chain are called respiration.		
5.241	Components	The electron transport system consists of	Components of the electron trnsport sys-	Procaryotic microorganisms have the

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		a series of molecules (flavoproteins, quinones, cytochromes) that are embedded in a cellular membrane. This series of molecules is referred to as the respiratory chain. These molecules have the ability to be both good electron acceptors and good electron donors. They are ordered in a sequence determined by their avidity in accepting electrons.	tem are much more variable among procaryotic organisms. The position of the flavin component (FAD) is in question and cytochrome *c* is not present in all bacteria. Those with certain cytochrome components give a positive oxidase test and this is an important diagnostic characteristic for certain bacterial groups.	molecules of the electron transport system associated with the plasma membrane. Eucaryotic microorganisms have these components in the inner mitochondrial membrane. If the components are extracted from the membrane, they will no longer function to transport electrons in a coordinated way.
5.242	Mechanism	The prime cellular donor of electrons to the electron transport system is NADH. When NADH provides an electron pair, the electron transport molecules are alternately reduced and reoxidized in a particular order as the electrons pass from one to the next. As these stepwise oxidation-reduction reactions occur, energy is released, and this energy can be conserved by oxidative phosphorylation (Microbial Physiology, Subtopic 5.25).	Branched pathways of electron transport are not uncommon in bacteria. Flavin enzymes can donate electrons directly to O_2, but H_2O_2 is formed instead of water. The peroxide is decomposed by catalase. The presence or absence of this enzyme is important in characterizing bacteria.	A typical electron transport chain is illustrated in Fig. 10.
5.243	Terminal Electron Acceptors	For the continuation of electron flow through this pathway, there must be some exogenous compound that will accept electrons from, and thus reoxidize, the last cytochrome in the chain.	If there is no terminal acceptor or if the transport is blocked at the last step, all of the electron transport components will become reduced and NADH cannot be reoxidized. If there are not alternate methods of reoxidizing NADH, metabolism will not continue.	
5.2431	Aerobic Respiration	Most microorganisms utilizing electron transport require oxygen to accept electrons from the final reduced cytochrome.	Obligate areobes are microorganisms that cannot grow and reproduce in the absence of molecular oxygen. They require oxygen as a terminal electron acceptor.	
5.2432	Anaerobic Respiration	A few types of bacteria can use oxidized inorganic ions as electron acceptors in the absence of oxygen. NO_3^- (nitrate) and SO_4^{2-} (sulfate) are the most common alternate electron acceptors.	Bacteria that use NO_3^- as a terminal electron acceptor (denitrifiers) will use oxygen preferentially. The conversion of soil nitrate (NO_3^-) to gaseous nitrogen products by denitrifying bacteria is one way that soils lose nitrogen. The use of SO_4^{2-} as a terminal electron acceptor is restricted to a specialized group of anaerobic organisms. H_2S is produced. Water-logged sediments with an	Nitrate reduction can be observed in the laboratory by culturing dentrifying bacteria under anaerobic conditions with NO_3^- added to the medium. NO_2^- may be produced, e.g., *E. coli*. Dentrifiers reduce NO_3^- to N_2 or other gaseous products, e.g., *Pseudomonas aeruginosa*. Winogradsky columns (Microbial Physiology, Subtopic 2.12) usually develop a good

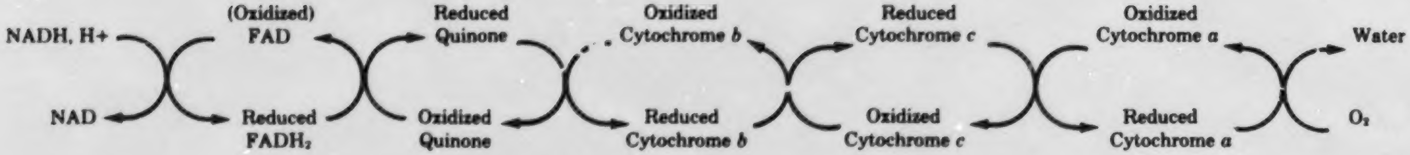

NADH, H+
NAD
(Oxidized)
FAD
Reduced
FADH$_2$
Reduced
Quinone
Oxidized
Quinone
Oxidized
Cytochrome *b*
Reduced
Cytochrome *b*
Reduced
Cytochrome *c*
Oxidized
Cytochrome *c*
Oxidized
Cytochrome *a*
Reduced
Cytochrome *a*
Water
O$_2$

FIG. 10. *Typical electron transport system.*

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			abundance of organic material and SO_4^{2-} (tideflats, for example) are noted for their H_2S-producing *Desulfovibrio* populations.	population of sulfate-reducing bacteria. It is possible to smell the H_2S produced in these cylinders or test for it with filter paper soaked in 5% lead acetate.
5.25	Substrate Level and Oxidative Phosphorylation	The principal method of conserving energy released during cellular oxidations is coupling the energy-yielding reaction to a reaction adding phosphate to a nucleoside diphosphate (Microbial Physiology, Subtopic 5.16). In substrate-level phosphorylation, the phosphate is transferred directly from a high-energy phosphorylated intermediate of metabolism to the nucleoside diphosphate. In oxidative phosphorylation, the energy released by the oxidative steps of the electron transport chain is conserved by some intermediary process and then is used to phosphorylate ADP to ATP. The energy released by moving one pair of electrons from NADH through the electron transport chain to oxygen is sufficient to phosphorylate three ADP molecules to three ATP molecules.	Although no one mechanism for oxidative phosphorylation has been proven, the chemiosmotic hypothesis of Mitchell seems best supported by available evidence. This presumes that energy released causes a flow of protons across the membrane and the proton gradient drives the phosphorylation. Each $2H^+$ equivalents transported results in phosphorylation of 1 ADP → 1 ATP. It has been difficult to demonstrate that 3 ATP are produced for each NADH oxidized in bacterial electron transport systems. Bacterial electron transport systems may be less efficient than mitochondrial electron transport systems (Microbial Physiology, Topic 6.0).	
5.26	Degradative or Catabolic Pathways	Cells break down organic molecules by series of enzyme-catalyzed reactions. A particular series of reactions is called a metabolic pathway.		
5.261	Glycolysis (Embden-Meyerhof Pathway)	Glycolysis is a pathway for degradation of glucose that is found in most microorganisms. There are nine reactions needed to convert glucose to two pyruvate molecules. $\text{Glucose} + 2\ \text{NAD}^+ + 2\ \text{ADP} + 2\ \text{Pi} \rightarrow 2\ \text{Pyruvate} + 2\ \text{NADH} + 2\ \text{ATP}$ There is one oxidative step in which	Glycolysis is essentially the same in microorganisms, plants, and animals. It is a cytoplasmic cellular process, and the enzymes are not membrane-bound. The intermediate substrates are all phosphorylated. The pathway can be divided into three phases. The preparative phase converts glucose to two three-carbon phosphorylated molecules. This requires two molecules of ATP. The oxidative phase converts the three-carbon molecules to the highly reactive 1,3-diphosphoglyceric acid (3-phosphoglyceroyl phosphate). This requires two	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		NAD$^+$ accepts a pair of electrons from a three-carbon intermediate. The ATP molecules are produced by substrate-level phosphorylation. The pyruvate and NADH generated have a different fate in fermentative organisms than in respiratory organisms.	molecules of NAD$^+$ and generates 2 NADH. The energy-transfer phase results in substrate-level phosphorylation of 4 ADP to 4 ATP. Two reactions occur whereby energy and phosphate are transferred directly from the 3-carbon phosphorylated high-energy intermediates to ADP (Fig. 11).	
5.262	Other Pathways	There are other routes by which sugars can be catabolized. For example, glucose can be catabolized to pyruvate by the pentose phosphate pathway and the Entner-Doudoroff pathway. Catabolic use of these pathways is restricted to certain bacterial groups.	A unique characteristic of procaryotic cells is the diversity of catabolic pathways that may be found in different species. This may be a reflection of the intensive investigation of this aspect of bacterial metabolism.	The pathway by which glucose is catabolized may be an important taxonomic characteristic of some microorganisms. Most members of the genus *Pseudomonas* characteristically use the Entner-Duodoroff pathway.
5.263	Fates of Pyruvate and NADH			
5.2631	Fate in Absence of Respiratory Pathways-Fermentation	Fermenting microorganisms are unable to carry out the reactions of electron transport either because they lack a terminal acceptor or because they lack the necessary enzymes or cofactors. They can grow and reproduce on the ATP produced by glycolysis or other anaerobic pathways, but, because NAD$^+$ is in very limited supply, they must be able to reoxidize the NADH produced to permit the anaerobic energy-yielding pathway to continue. Fermentative microorganisms utilize pyruvate or other products directly or indirectly as recipients for electrons from NADH. Thus, much of the pyruvate carbon can be found in the reduced organic molecules (fermentation products) excreted by fermenting organisms.	Because the energy yield per molecule of substrate utilized is low, a fermenting microorganism must catabolize more molecules of substrate than a respiring microorganism for the same yield of growth and reproduction. In fermentations there is no exogenous electron acceptor, so the average oxidation level of the fermentation products must equal that of the substrates utilized. This requirement, and the fact that no oxygen is available to carry out oxidative bond cleavage, limits the range of substrates that can be fermented. In general, compounds more oxidized than pyruvate or more reduced than the aliphatic amino acids do not serve as substrate for fermentations.	It is possible to identify certain taxonomic groups of bacteria by their fermentation products. The identification of anaerobic pathogenic bacteria by gas chromatographic analysis of their volatile fermentation products may become an important clinical technique. The Voges-Proskauer test for the presence of acetoin is frequently used in identification of enteric bacteria. Two fermentative pathways are illustrated in Fig. 12. For additional examples consult microbiology and biochemistry textbooks.
5.2632	Fate of Pyruvate and NADH in Organisms Utilizing Respiratory Pathways	In microorganisms with a functional electron transport system, NADH can be readily reoxidized by this system. Pyruvate can be completely oxidized to CO_2 by the tricarboxylic acid cycle.		
5.26321	Oxidative Decarboxylation of Pyruvate	Pyruvate is converted to acetyl CoA which then enters tricarboxylic acid cycle.	This reaction is carried out by a multienzyme complex containing three different en-	Figure 13.

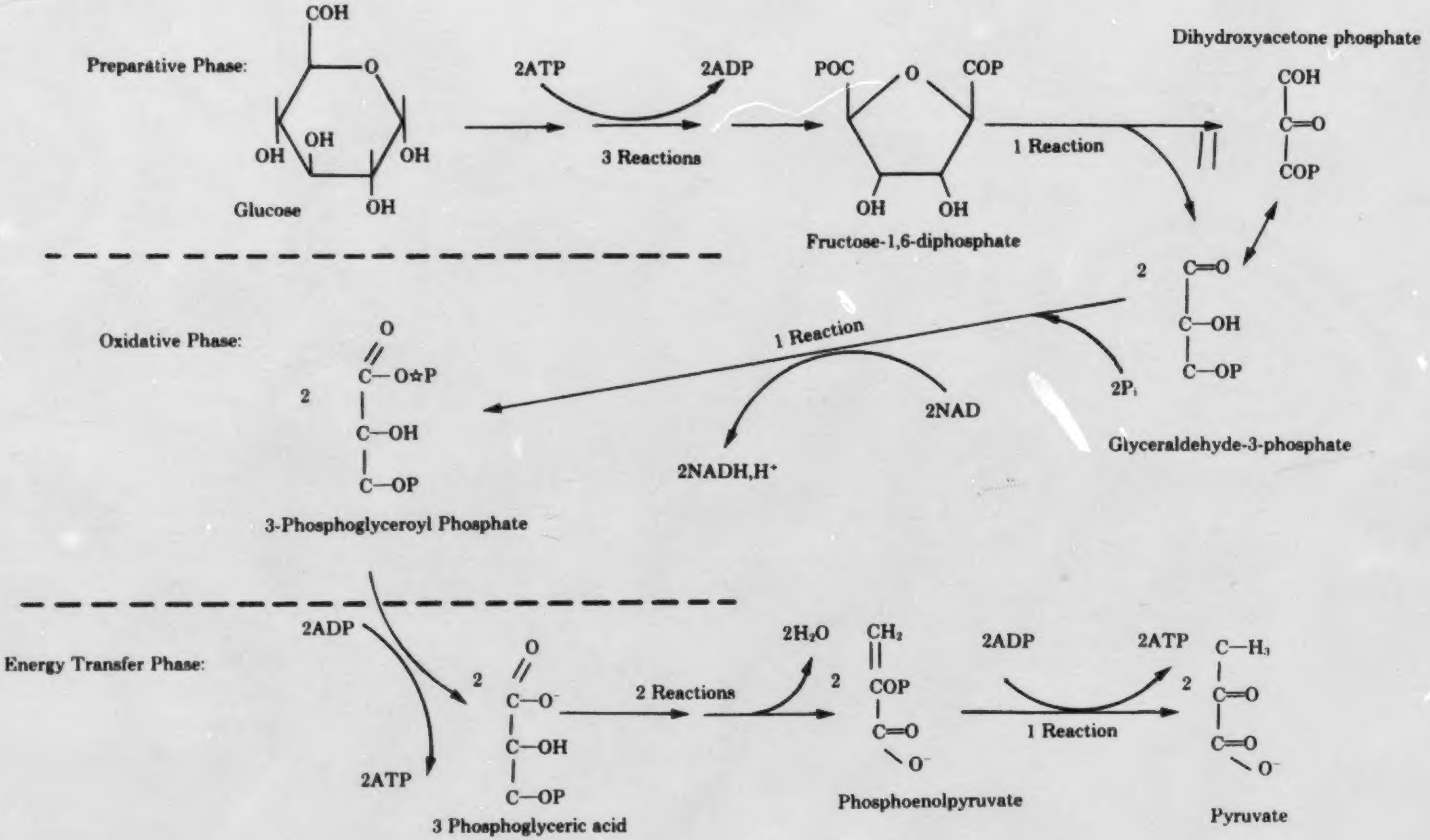
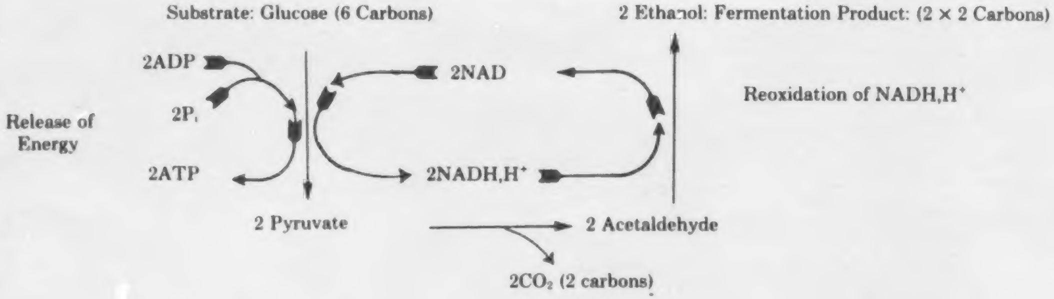
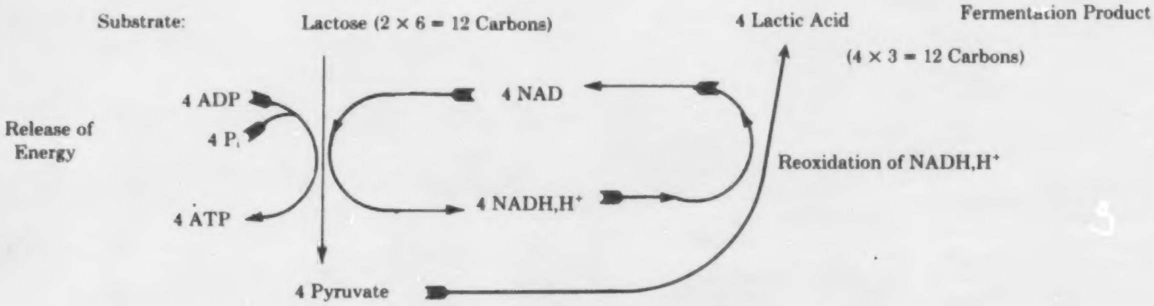

Preparative Phase:
COH
O
OH
OH
OH
OH
Glucose
2ATP
2ADP
3 Reactions
POC
O
COP
OH
OH
Fructose-1,6-diphosphate
1 Reaction
Dihydroxyacetone phosphate
COH
C=O
COP
2
C=O
C—OH
C—OP
2Pi
Glyceraldehyde-3-phosphate
Oxidative Phase:
1 Reaction
2NAD
2NADH,H+
O
2
C—O☆P
C—OH
C—OP
3-Phosphoglyceroyl Phosphate
Energy Transfer Phase:
2ADP
2ATP
O
2
C—O-
C—OH
C—OP
3 Phosphoglyceric acid
2 Reactions
2H2O
2
CH2
COP
C=O
O-
Phosphoenolpyruvate
2ADP
2ATP
1 Reaction
2
C—H3
C=O
C=O
O-
Pyruvate

Fig. 11. *Glycolysis.*

The reduced organic molecules produced by fermenting microorganisms are of great commercial and diagnostic importance. Ethanol is produced mainly as a product of yeast fermentation.

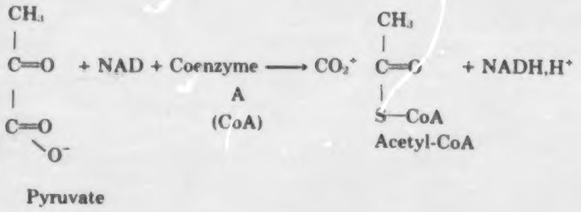

Substrate: Glucose (6 Carbons)
2 Ethanol: Fermentation Product: (2 × 2 Carbons)
2ADP
2P$_i$
Release of Energy
2ATP
2NAD
2NADH,H$^+$
Reoxidation of NADH,H$^+$
2 Pyruvate
2 Acetaldehyde
2CO$_2$ (2 carbons)

Lactic acid, a product of the fermentation of milk sugar (lactose) by lactobacilli and streptococci, is important in production of cheese and yogurt.


Substrate:
Lactose (2 × 6 = 12 Carbons)
4 Lactic Acid
Fermentation Product
(4 × 3 = 12 Carbons)
4 ADP
4 P$_i$
Release of Energy
4 ATP
4 NAD
4 NADH,H$^+$
Reoxidation of NADH,H$^+$
4 Pyruvate

In the clinical laboratory, bacterial isolates may be identified on the basis of fermentation substrates and products.

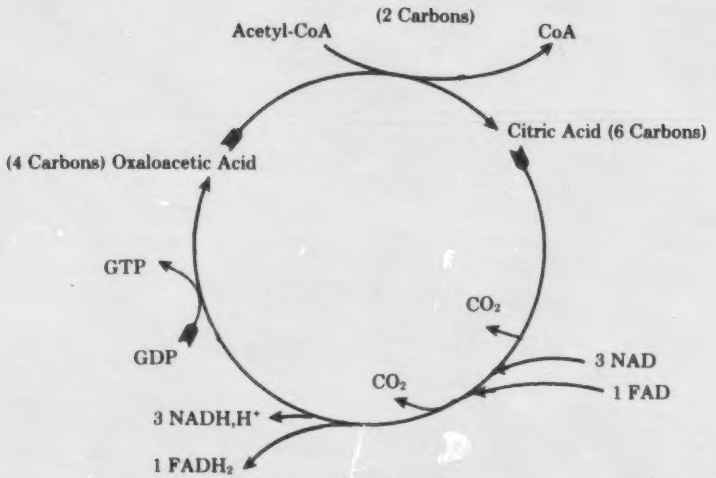
FIG. 12. *Examples of fermentative pathways.*



$$\begin{array}{c} CH_3 \\ | \\ C{=}O \\ | \\ C{=}O \\ \diagdown O^- \\ \text{Pyruvate} \end{array} + NAD + \begin{array}{c}\text{Coenzyme}\\ \text{A}\\ \text{(CoA)}\end{array} \longrightarrow CO_2 + \begin{array}{c} CH_3 \\ | \\ C{=}O \\ | \\ S{-}CoA \\ \text{Acetyl-CoA} \end{array} + NADH,H^+$$

FIG. 13. *Oxidative decarboxylation of pyruvate.*

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	Coenzyme A (CoA) is essential to this reaction and serves to carry an activated acetyl group into the cycle.	zymes and five cofactors. ATP concentration regulates activity of the enzyme complex.	
5.26322 Tricarboxylic Acid Cycle (Krebs' Cycle) (Citric Acid Cycle)	The further oxidation of acetyl CoA is dependent on enzymes of the tricarboxylic acid cycle. These enzymes are associated with the cytoplasmic membrane of procaryotic cells and are in the mitochondria of eucaryotic cells.	The tricarboxylic acid cycle has more than just a catabolic function. Many of the intermediates are starting material for synthesis of important cell components. Anaerobes that do not use the cycle for respiratory metabolism may still have many of the cycle enzymes. These have biosynthetic function. When tricarboxylic acid intermediates are removed for biosynthesis, the cycle can be kept in operation by synthesizing new oxaloacetate. The reactions responsible for synthesizing oxaloacetate from pyruvate, phosphoenol pyruvate or acetyl CoA are called anaplerotic or replenishing reactions. Because of the central position of many of the tricarboxylic acid intermediates in metabolism, an expanded version of the tricarboxylic acid cycle is presented in Fig. 15.	Figures 14 and 15.
5.264 Pathways for Degradation of Other Substrates	Microorganisms can use a wide range of carbon compounds as sources of carbon and energy. Series of degradation reactions, often catalyzed by inducible enzymes (Microbial Genetics, Subtopic 6.1), result in products that can enter the glycolytic or tricarboxylic acid cycle pathways.	Some synthetic molecules are very resistant to microbial degradation. DDT is one of these recalcitrant molecules. Pesticide chemical / Approximate half-life (yr) Chlordane 2–4 DDT 3–10 Dieldrin 1–7 Heptachlor 7–12	Microorganisms vary greatly in their ability to degrade carbon compounds. These need not be water soluble, e.g., crude oil. Microorganisms able to degrade large molecules often excrete the degradative enzymes into the medium containing the substrate. These excreted enzymes are called exoenzymes and can be detected by growing the microorganisms on solid medium containing the substrate and observing substrate disappearance around the areas of microbial growth.
5.2641 Carbohydrates	Enzymes catalyze removal of monosaccharide or disaccharide units from polysaccharides. Disaccharides are broken into two monosaccharides. Glucose and fructose enter the glycolytic pathway directly. Most other monosaccharides can be converted to either glucose or fructose.	Certain polysaccharides such as starch and glycogen are readily degraded by exoenzymes secreted by many microorganisms. The breakdown of cellulose is limited to a few groups of bacteria, some fungi, and a few protozoan types.	A solid nutrient agar medium containing starch can be inoculated with an amylase-producing microorganism; after incubation and growth of the organism, flood the plate with a 3% iodine solution and note the starch-free area around the microbial growth. An example of an amylase-producing bacterium is *Bacillus subtilis*.



Acetyl-CoA
(2 Carbons)
CoA
Citric Acid (6 Carbons)
(4 Carbons) Oxaloacetic Acid
GTP
GDP
CO₂
3 NAD
1 FAD
CO₂
3 NADH,H⁺
1 FADH₂

A summary of the cycle. (i) Acetyl-CoA reacts with a 4-carbon dicarboxylic acid (oxaloacetic acid) to produce a 6-carbon tricarboxylic acid (citric acid). CoA is released. (ii) Citric acid undergoes a series of reactions which convert it back to oxaloacetate, thus completing one cycle. In each cycle four oxidative reactions occur (three NADH,H$^+$ and one FADH$_2$ produced), 2CO$_2$ are released, oxaloacetate is regenerated, and one substrate-level phosphorylation occurs (GTP). (iii) The 3 NADH,H$^+$ and 1FADH$_2$ donate electrons to the electron transport chain and ATP is produced by oxidative phosphorylation (see 5.24).

FIG. 14. *Tricarboxylic Acid cycle summary.*

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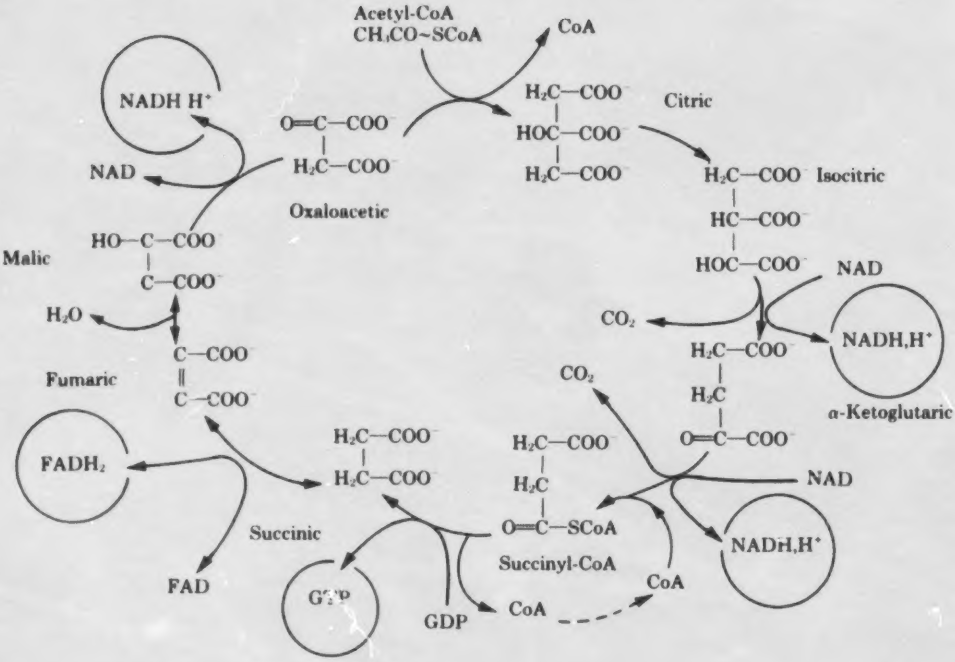
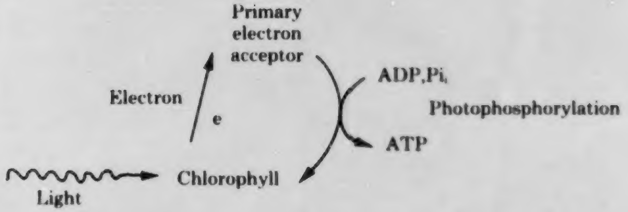

Acetyl-CoA
CH₃CO~SCoA
CoA
Citric
Isocitric
NADH H⁺
NAD
O=C—COO⁻
H₂C—COO⁻
Oxaloacetic
H₂C—COO⁻
HOC—COO⁻
H₂C—COO⁻
H₂C—COO⁻
HC—COO⁻
HOC—COO⁻
NAD
Malic
HO—C—COO⁻
C—COO⁻
H₂O
CO₂
NADH,H⁺
Fumaric
C—COO⁻
C—COO⁻
H₂C—COO⁻
H₂C
O=C—COO⁻
α-Ketoglutaric
CO₂
FADH₂
H₂C—COO⁻
H₂C—COO⁻
H₂C—COO⁻
H₂C
O=C—SCoA
NAD
Succinic
Succinyl-CoA
NADH,H⁺
FAD
GTP
GDP
CoA
CoA

FIG. 15. *Tricarboxylic acid cycle.*

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
5.2642	Lipids	Lipids are cleaved into their component parts. Fatty acids and other hydrocarbons are degraded by oxidative reactions to acetyl CoA which enters the tricarboxylic acid cycle. Glycerol can enter the glycolytic pathway.	Many of the normal mammalian skin bacteria, e.g., diptheroids, are good at degrading fatty acids. The odor produced by various mammals, including humans, is in part generated by the action of skin bacteria on skin fatty acids.	The breakdown of fatty acids and hydrocarbons usually requires molecular oxygen. This explains the environmental persistence of these compounds under anaerobic conditions. Several media can be used to detect bacterial ability to degrade lipids, e.g., media containing egg yolk or Tween 80 (sorbitan mono-oleate polyoxyethylene). A precipitate around the colony indicates lipolytic ability. Various members of the genus *Clostridium* may be identified by their phospholipase activity.
5.2643	Proteins	Proteases degrade proteins into amino acid units. Amino acids are converted to pyruvate, acetyl groups, or tricarboxylic acid cycle intermediates.	Some of the proteolytic exoenzymes formed by the members of the genus *Bacillus* have found commercial use as additives to washing products. They degrade protein-containing stains like blood and gravy.	Many microorganisms are able to degrade proteins. A common laboratory test for ability to hydrolyze protein involves inoculating an organism onto solid gelatin medium and noting liquefaction after growth.
5.3	Inorganic Compounds as Energy Sources	A small number of procaryotic species can oxidize reduced inorganic compounds and use the energy released by these oxidations to form ATP. The electrons from the oxidative reactions enter the electron transport system (Microbial Physiology, Subtopic 5.24), and ATP is produced by oxidative phosphorylation (Microbial Physiology, Subtopic 5.25). These organisms, called lithotrophs, usually obtain their carbon from CO_2 and thus are autotrophs as well.	Usually lithotrophs are quite specific for a given inorganic material. For example, sulfur oxidizers do not oxidize reduced nitrogen compounds. For most lithotrophs, this mode of existence is an obligate one. They do not oxidize organic molecules, and, in fact, some will not grow in the presence of moderate concentrations of organic materials. The hydrogen bacteria, however, can also obtain energy by oxidizing organic molecules.	Media made with a particular reduced inorganic compound as an energy source and CO_2 as a carbon source will develop a population of lithotrophs when inoculated with soil or water and incubated in the dark. Some of the reduced inorganic molecules that can serve as energy sources are: H_2 (hydrogen oxidizers); NO_2^- (nitrite oxidizers); H_2S, S, $S_2O_3^{=}$ (sulfur oxidizers); NH_3 (ammonia oxidizers); Fe^{2+} (iron oxidizers).
5.4	Light as an Energy Source	Organisms able to use light as an energy source are called phototrophs. Phototrophs convert light energy to chemical bond energy by some light-absorbing pigment—usually a form of chlorophyll. Chlorophyll is oxidized by light of certain wavelengths. The electrons removed from chlorophyll are then high-energy electrons. They enter an electron transport system and ATP is produced (Microbial Physiology, Subtopic 5.24).	There are procaryotic phototrophs, e.g., photosynthetic bacteria and cyanobacteria (blue-green algae) and eucaryotic microbial phototrophs (algae). Nearly all the organic chemical energy on the earth is derived from light energy trapped by phototrophs. Microbial phototrophs (predominantly marine) are responsible for about 50% of the global conversion of light energy to chemical bond energy.	

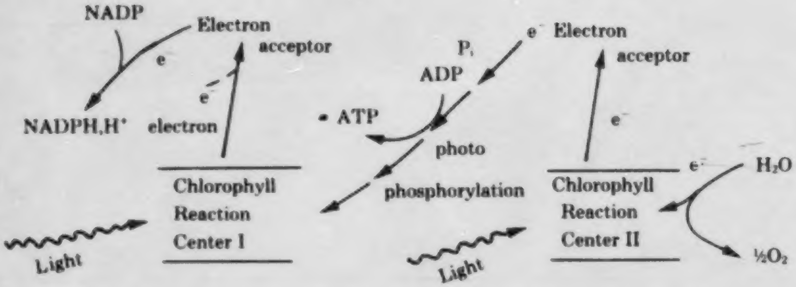
TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
5.41	Anoxygenic Photosynthesis	Photosynthetic bacteria (other than cyanobacteria) carry out photosynthesis only in the absence of oxygen. Their photosynthesis does not produce O_2. In these organisms, electrons released from chlorophyll provide energy for synthesis of ATP (Microbial Physiology, Subtopic 6.1232). Electrons reducing $NADP^+$ to the NADPH needed to fix CO_2 are derived from other sources.	Some bacterial photosynthesizers (purple and green sulfur bacteria) require reduced sulfur compounds as a source of electrons to reduce $NADP^+$. This limits their distribution to illuminated anaerobic areas which have a source of reduced sulfur compounds. Electron flow in a photosynthetic bacterial system is often described as cyclic and can be diagrammed as in Fig. 16.	
5.42	Oxygenic Photosyntheses	In cyanobacteria (blue-green algae) and eucaryotic algae (as well as all higher plants), electrons from chlorophyll provide energy for syntheses of ATP and serve as a reductant for $NADP^+$. Electrons from water reduce chlorophyll, and the oxygen from the water is released as O_2.	Cyanobacteria (blue-green algae) do not have chloroplasts. Although the reactions of their oxygenic light reaction are quite similar to those occurring in eucaryotic photosynthesizers, all of the components of the photosystem reside in membranous sheets or sacs in the cytoplasm. Some investigators think that chloroplasts may have evolved from cyanobacterial symbionts residing in a larger host cell. The oxygen-producing noncyclic photophosphorylation and photoreduction system can be diagrammed as in Fig. 17.	
5.5	Anabolism	Anabolism is the sum of the cellular chemical reactions that produce the organic molecules necessary for maintenance, growth, and reproduction of the organism.		
5.51	CO_2 Fixation in Autotrophs	The conversion of CO_2 into the carbon compounds of cell material requires energy and reducing power.	The primary reaction in autotrophic CO_2 fixation is the binding of CO_2 to the five-carbon sugar ribulose 1,5-diphosphate. This results in production of two phosphoglyceric acid molecules (each three carbons). These can be used as starting material to synthesize the carbon compounds needed by the autotroph.	CO_2 fixation (the Calvin cycle) was first investigated in green algae. The path of incorporated ^{14}C-labeled CO_2 was observed in photosynthesizing organisms. It was later discovered that this pathway is used by almost all autotrophs.
5.52	Relationship Between Anabolism and Catabolism	Catabolic metabolism provides sources of energy (ATP), reducing power (NADH and NADPH), and carbon skeletons for biosyntheses. Withdrawal of intermediates from catabolic pathways diminishes the net ATP yield. Control mechanisms function so		

7


Primary
electron
acceptor
Electron
e
ADP,Pi,
Photophosphorylation
ATP
Light
Chlorophyll

Chlorophyll acts as the donor and the acceptor of the electrons.

FIG. 16. *Cyclic electron flow in phototrophic bacteria.*


NADP
Electron
acceptor
e
e
NADPH,H⁺
electron
Light
Chlorophyll
Reaction
Center I
ATP
ADP
P_i
e⁻
photo
phosphorylation
Electron
acceptor
e⁻
Chlorophyll
Reaction
Center II
Light
e⁻
H₂O
½O₂

[center]

FIG. 17. *Noncyclic electron flow in cyanobacteria and eucaryotic photosynthesizers.*

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		that anabolic processes (biosynthetic pathways) are maximized when the ATP supply is high and catabolic processes predominate when ATP supply is low.		
5.53	Synthesis of Monomers	Microorganisms can synthesize most of the small molecules (monomers) needed for growth. The starting carbon compounds for these pathways come from intermediates in catabolic pathways. Monomers not synthesized must be provided in the medium (Microbial Physiology, Subtopic 2.25).		
5.531	Hexose Phosphates	Oxaloacetate, a tricarboxylic acid cycle intermediate, can be used as starting material for hexose synthesis. Since oxaloacetate can be synthesized from pyruvate and CO_2 and from other tricarboxylic acid cycle intermediates, and in certain organisms from acetyl CoA, any compound that can be converted to these will also serve as a precursor of hexoses (Fig. 18).	Microorganisms able to synthesize hexoses from acetyl CoA utilize a special pathway, the glyoxylate bypass, in conjunction with the tricarboxylic acid cycle. Acetyl CoA can be condensed with glyoxylate to form malate. This permits accumulation of tricarboxylic acid cycle intermediates for biosynthesis.	
5.532	Pentose Phosphates	Deoxyribose and ribose are needed for synthesis of nucleotides. Ribose is synthesized by the pentose phosphate pathway. This pathway, often called the hexose monophosphate shunt, involves an oxidative decarboxylation of hexose phosphate to a pentose phosphate. The NADPH produced during the oxidative steps of this pathway is important in providing electrons for reductive steps in biosynthetic pathways. Ribose can only be converted to deoxyribose when it is incorporated in a nucleotide.	The pentose phosphate pathway has two stages. The oxidative portion converts glucose 6-phosphate to ribose 5-phosphate. The rearrangement portion converts six pentose phosphates to five hexose phosphates. Depending on the biosynthetic needs of the organisms one or both portions may function.	
5.533	Lipids	There is considerable diversity in the lipids synthesized by microorganisms. Fatty acids, phospholipids, carotenoid pigments, quinones, poly-β-hydroxybutyrate, and chlorophyll can be synthesized by various procaryotes. Eucaryotic microorganisms synthesize a different range of fatty acids	For fatty acid synthesis the general pathway involves successive addition and reduction of two carbon units (derived from acetyl CoA) to a growing fatty acid chain held on a carrier protein. Double bonds are either left in during synthesis or added when the chain is finished.	The types of fatty acids synthesized by a particular microbial group are often characteristic of that group and may be identified by gas or thin-layer chromatography. It may be possible to determine the relative amounts of eucaryotic and procaryotic microbial biomass in environmental samples

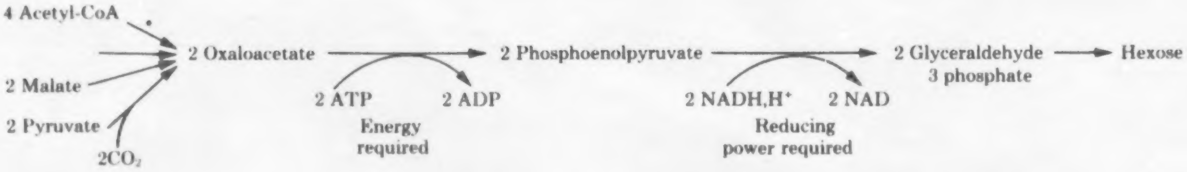

4 Acetyl-CoA
2 Malate
2 Pyruvate
2CO$_2$
2 Oxaloacetate
2 ATP
2 ADP
Energy required
2 Phosphoenolpyruvate
2 NADH,H$^+$
2 NAD
Reducing power required
2 Glyceraldehyde 3 phosphate
Hexose

FIG. 18. *Hexose synthesis. Once glucose-P or fructose-P is synthesized these sugars can be converted to a wide range of amino sugars, sugar acids, and deoxysugars. Often sugar conversions are carried out while the sugar is in an activated form. Example: UDP glucose ↔ UDP galactose. *Glyoxylate bypass is not found in all organisms.*

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	than procaryotic microorganisms. Eucaryotes usually synthesize considerable amounts of sterol as well. Glycerol phosphate needed for phospholipid synthesis is synthesized from dihydroxyacetone phosphate, an intermediate of the glycolytic pathway. Fatty acids are synthesized from acetyl CoA. Glycerol phosphate, fatty acids, and additional components are combined to produce phospholipids. Other types of lipids are synthesized from CO_2 and two carbon units derived from acetyl CoA.	Bacteria, with the exception of cyanobacteria, do not synthesize fatty acids with more than one double bond.	by analyzing the characteristics of extracted lipids.
5.534 Amino Acids	The addition of an amino group to pyruvate, oxaloacetate, or α-keto-glutarate is the starting point for the biosynthesis of most amino acids.	Some microorganisms can synthesize all 20 amino acids required for protein synthesis and some cannot. The amino acids synthesized by short pathways are the most universally synthesized. An example would be glutamate. The pathways for synthesis of some other amino acids are long and expensive in terms of ATP and reducing power (NADH). These amino acids are required as growth factors by the organisms unable to synthesize them. Some of these longer pathways are branched, giving rise to several amino acids. An example might be the isoleucine-valine pathway.	
5.535 Nucleoside Monophosphates	Small chemical groups containing carbon and nitrogen derived primarily from amino acids are assembled into the purine and pyrimidine rings. The purine ring is assembled starting with an activated form of ribose phosphate. In the case of pyrimidine ribonucleotide, the pyrimidine ring is synthesized and then attached to the activated ribose phosphate (Fig. 19).		
5.54 Synthesis of Polymers	Polymers are synthesized by condensation of monomers. Energy is required for this condensation. Monomers are activated by a		

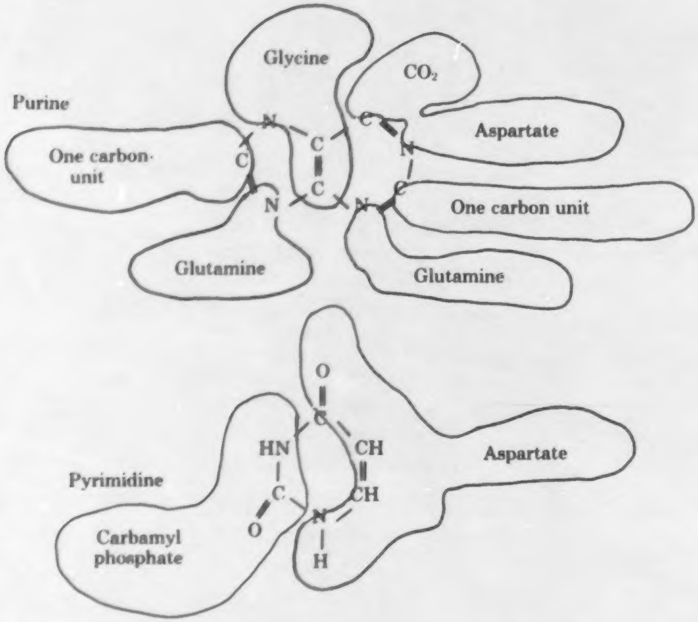

Purine
Glycine
CO₂
Aspartate
One carbon unit
One carbon unit
Glutamine
Glutamine
N
C
C
C
N
N
C
N
C
N
Pyrimidine
O
C
HN
CH
CH
C
N
O
H
Aspartate
Carbamyl phosphate

FIG. 19. *Origin of carbons and nitrogens in purines and pyrimidines. The purine ring is synthesized from seven units derived from six different sources. It takes seven ATP molecules to build a purine ring. The pyrimidine ring is synthesized from two units. One of these units is carbamyl phosphate, an activated compound.*

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	reaction with a high-energy compound (usually a nucleoside triphosphate like ATP).		
5.541 Polysaccharides	Monosaccharides are added one at a time to a growing polysaccharide. Each monomer is first activated by reacting with a nucleoside triphosphate. The monosaccharide can be transferred from this active unit to the end of an existing polysaccharide chain without further expenditure of energy (Fig. 20).		
5.542 Informational Polymers: DNA, RNA, and Protein	These template-dependent polymers are synthesized from their respective monomeric units by mechanisms described in Microbial Genetics, Subtopics 2.13, 2.23, and 2.33.	Bacteria have been extraordinarily useful as experimental systems in investigations of the synthesis of DNA, RNA, and protein.	
6.0 Membrane Phenomena			
6.1 Membranes and Energy	Membranes are energy transducers. That is, within the membrane structure, exergonic reactions occur and are coupled to endergonic activities. The membrane itself is essential for coupling.		
6.11 Membrane Semipermeability	Membranes are semipermeable. They permit the free passage of water in either direction, but restrict the movement of ions and small organic molecules. These may enter and leave only at certain points. Transport of such substances is under tight cellular control.		
6.111 Gradients	Often the concentration of a substance may be much greater on one side than on the other. When this occurs, a gradient is said to exist.		
6.112 Movement in the Gradient	When molecules of a substance move from an area where they are more concentrated to one where they are less concentrated, they move down the gradient. This can occur spontaneously without the expenditure of energy. In fact, such movement is exer-		

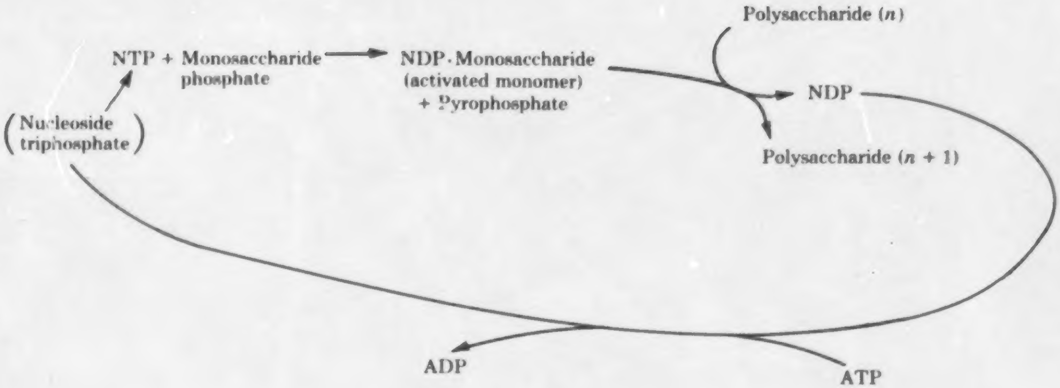
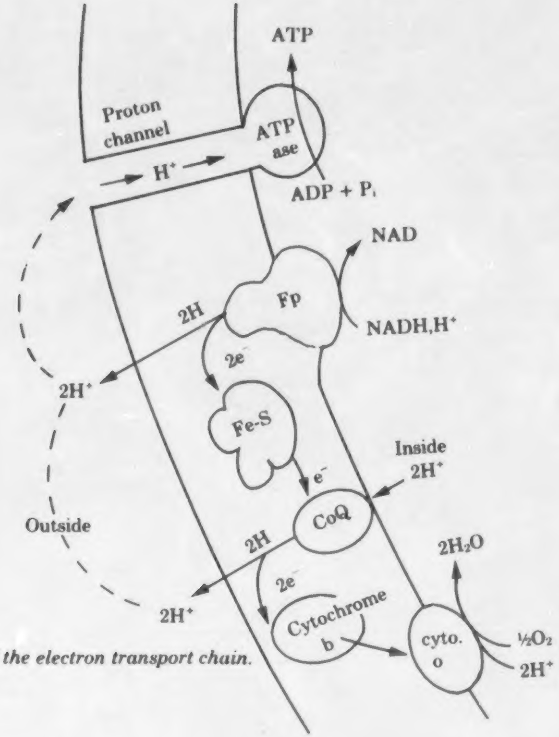

Polysaccharide (*n*)
NTP + Monosaccharide phosphate
NDP·Monosaccharide (activated monomer) + Pyrophosphate
NDP
Polysaccharide (*n* + 1)
(Nucleoside triphosphate)
ADP
ATP

FIG. 20. *Synthesis of polysaccharides.*

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	gonic—it releases energy. The membrane prevents this from happening in a random fashion. When molecules move from a less to a more concentrated area, they are moving against the gradient. This requires the expenditure of energy, which is endergonic.		
6.113 Gradients as Energy Sources	Since movement of substances down gradients releases energy, a gradient, like stored water water behind a dam, is a source of energy the cell can tap.		
6.114 Proton Gradients	Several cell processes generate proton gradients where the concentration of protons (H^+) exterior to the cell is greater than that within. Since the exterior protons, if allowed to reenter the cell, act as an energy source, the gradient and resulting membrane potential is referred to as proton motive force (PMF).		
6.115 Proton Reentry	The membrane is not permeable to protons except at certain points (proton channels). Reentry at those points is coupled to ATP synthesis. Membrane-bound Mg^{2+}-dependent adenosine triphosphatase (ATPase) is responsible for synthesis of ATP as protons move across the membrane. Proton reentry can also be coupled to transport of small molecules.	Part of the ATPase complex is a proton channel. Artificial membranes containing this section are proton permeable.	
6.12 Means of Producing Proton Gradients	Proton gradients are produced in several different ways in various organisms under specific conditions. Three discussed below are electron transport, ATP hydrolysis, and light-driven transport.	Production of proton gradients requires closed membrane systems. Broken cells or organelles no longer maintain proton gradients or synthesize ATP. **Hinkle, D. C., and R. E. McCarty.** 1978. How cells make ATP. Sci. Am. **238:**104-123.	
6.121 Electron Transport	(See Microbial Physiology, Subtopic 5.25 for information on electron transport.) The chemiosmotic hypothesis explains the means by which electron transport (exergonic) is coupled to ATP synthesis (ender-	Figure 21 represents a possible interpretation of an *E. coli* proton translocation system. ATP synthesis occurs during reentry of the protons.	


ATP
Proton channel
ATP ase
H⁺
ADP + Pᵢ
NAD
Fp
2H
NADH,H⁺
2e⁻
2H⁺
Fe-S
Inside 2H⁺
e⁻
CoQ
Outside
2H
2H₂O
2e⁻
2H⁺
Cytochrome b
cyto. o
½O₂
2H⁺

F_p, Flavoprotein; Fe-S, non-heme iron protein; CoQ, quinone component of the electron transport chain.

FIG. 21. *Proton translocation and ATP synthesis.*

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		gonic) within the framework of a membrane. It states that when certain electron carriers are reoxidized, protons are extruded through the membrane. Thus electron transport results in a proton gradient being established across the membrane. The reducing power of the electrons is converted to PMF. This is the primary effect of electron transport.		
6.1211	Oxidative Phosphorylation	The protons are allowed to reenter through a channel where the PMF may be harnessed to ATP synthesis. This is the secondary effect of electron transport. Membrane-bound ATPase is essential to ATP synthesis.		
6.122	ATP Hydrolysis	The hydrolysis of ATP to ADP and inorganic phosphate (P_i) releases energy to pump protons out of the cell. Membrane-bound ATPases have two functions. They may break down ATP to establish proton gradients and they may synthesize ATP as protons reenter (Microbial Physiology, Subtopic 6.121). Cytoplasmic reactions (substrate-level phosphorylation) may provide the ATP necessary to produce proton gradients. This permits bacteria lacking electron transport systems to generate the PMF needed for active transport and motility.		
6.123	Light-Driven Proton Transport	Two systems of light-driven proton transport exist. One is the system found in halobacteria; the other is the light-dependent electron transport found in photosynthetic procaryotes and eucaryotes.		
6.1231	*Halobacterium* (purple membrane)	The halobacteria synthesize a purple membrane protein when they are grown in light without adequate oxygen. When light is absorbed by the pigment, the pigment is bleached and protons are extruded from the cell. A proton gradient is established and per-	**Stoeckenius, W.** 1976. The purple membrane of salt-loving bacteria. Sci. Am. **234**: 38–57.	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	sists as long as the cells are illuminated. This proton gradient allows the cells to synthesize ATP.		
6.1232 Light-Dependent Electron Transport	The ATP generated by the light reaction of photosynthesis is thought to be a result of the production of proton gradients across chlorophyll-containing membranes. Light striking chlorophyll results in vectorial electron transport reactions which in turn cause proton movement across the membrane. This generation of PMF and subsequent movement of protons back across the membrane through proton channels controlled by an ATPase leads to synthesis of ATP.	**Miller, K. R.** 1979. The photosynthetic membrane. Sci. Am. **241:**102–113.	
6.2 Biological Activities of Membranes	Two important functions of membranes are transport of small molecules and provision of energy for flagellar rotation in procaryotes.		
6.21 Transport of Small Molecules	Except for water, ammonia, gases, and some lipid-soluble molecules, metabolites move across membranes by specific energy-requiring transport systems. There are three basic types of transport in bacteria: transport driven by proton circulation, transport driven by ATP, and group translocation.	*E. coli* may produce over 100 distinct transport systems. These are all genetically coded and many are inducible (synthesized only in the presence of the substance to be transported).	
6.211 Proton-Driven Transport	These systems involve specific membrane proteins that act as carriers. The proton gradient (PMF) provides the energy and the direction of transport.		
6.2111 Symport: Transport with the Down-Gradient Movement of Protons	Certain molecules or ions are cotransported with one or more protons. The specific carrier binds both proton(s) and its transportee molecule and by some conformational change moves them to the other side of the membrane. The coupling of the down-gradient (exergonic) movement of protons provides the energy for the up-gradient (endergonic) movement of the transported molecule.	The lactose transport protein M of *E. coli* is an example of a symport carrier. One or perhaps two protons move to the inside of the cell with each lactose molecule.	

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
6.2112	Antiport: Transport Against the Down-Gradient Movement of Protons	When protons move from a higher to a lower concentration they provide energy for movement of some ions and molecules in the opposite direction. For example Na^+ and Ca^{2+} are moved out by the carriers that move protons in.	Antiport-produced Na^+ gradients may be used by the cell to drive influx of other molecules. Melibose and glutamate may be cotransported with Na^+ by *E. coli*.	
6.212	ATP-Driven Systems	These transport systems are commonly found in gram-negative bacteria. They have a periplasmic binding protein plus a specific membrane carrier. In the presence of ATP these systems can transport molecules so that the internal concentration far exceeds the external concentration. Systems such as this also exist in gram-positive bacteria, but the mechanism is not quite as clear.	Some sugars, amino acids, phosphate, and K^+ may be transported by such systems.	
6.213	Group Translocation	These systems catalyze a specific enzymatic reaction as the molecules are transported. The best known system depends on phosphorylation of sugars with phosphoenolpyruvate as a phosphate donor (phosphotransferase system).	The phosphotransferase system responsible for transporting glucose is found in many anaerobic and facultatively anaerobic bacteria, e.g., *E. coli*, but does not seem to occur in strict aerobes like *Pseudomonas* and *Mycobacterium*.	
6.22	Provision of Energy for Flagellar Rotation			
6.221	Structure of Flagella	Bacterial flagella are rigid helical structures composed of protein subunits. Each flagellum has a hook-shaped structure at its base. Two disks on the end of this hook are associated with the plasma membrane.	Flagella are good antigens and are responsible for the H antigenic determinants of gram-negative cells.	Flagella can be disaggregated into individual protein subunits. Given proper conditions these units will self-assemble into flagellar structures.
6.222	Function of Flagella	Flagella are rotated by a force applied through the membrane. Energy for this turning is generated by the proton circulation of the plasma membrane (PMF). Flagella rotating in one direction propel the cell in a straight line (a run). Reversal of rotation causes a disruption of this run (a tumble or twiddle).		Certain modified bacteria can be "tethered" to glass slides by antibody to their flagella. When the flagellum is unable to turn, the rotation force results in the cell body turning. This can be observed under the microscope and the rotation direction can be noted.
6.223	Chemotaxis	Bacteria are attracted to some chemical substances and are repelled by others. Peri-	The rotation direction and frequency of change of direction of "tethered" bacteria	One test for chemotaxis involves using a capillary with an attractive or repellent sub-

TOPICS AND SUBTOPICS

A. ESSENTIAL INFORMATION

plasmic proteins, the same ones involved in transport (Microbial Physiology, Subtopic 6.212), are involved in sensing concentration gradients of attractants or repellents. The plasma membrane is affected by this sensory binding of molecules. If the bacterium is swimming into a higher concentration of an attractant, the run will be prolonged. If it is swimming into a lower concentration, the flagellar rotation direction will reverse more frequently and swimming direction will change until an up-gradient swimming direction is resumed.

B. ENRICHMENT INFORMATION

(Microbial Physiology, Subtopic 6.222) can be observed when potential attractant or repellent chemicals are added to the slide preparation.

C. PRACTICAL ACTIVITIES

stance in it. The capillary is put into a suspension of bacteria. After a designated time the number of bacteria in the capillary is compared with the number in a control capillary without attractant or repellent.

Section IV. Microbial Genetics

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TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
1.0 General Introduction			
1.1 Definition of Genetics	Genetics is the study of the means by which hereditary information of cells is stored, expressed, and modified and the mechanisms by which this information is transmitted to other cells in the population and to future generations of organisms.		
1.2 Novel Features Making Microbes Useful Tools for Genetic Studies	Microorganisms have short generation times. Very large populations of almost identical cells can be produced asexually from a single cell in a short time.	Generation times determined for some typical bacteria growing in liquid media under optimum conditions are as follows: *Beneckea natriegens* 9 min *Escherichia coli* 17 min *Staphylococcus aureus* 30 min *Mycobacterium tuberculosis* ~800 min The time required for one cell to give rise to 10^9 cells under optimum conditions: *B. natriegens* 4.5 h *E. coli* 8.5 h *S. aureus* 15 h *M. tuberculosis* ~400 h	For microorganisms reproducing by binary fission, generation time can be determined by inoculating a culture with a known number of cells, determining the population size at a later time, and applying the formula: $\frac{t_1 - t_0}{3.3 \log_{10} b_1 - \log_{10} b_0}$ where: G = generation time t_0 = time at first measurement t_1 = time at second measurement b_0 = number of cells at beginning of time period b_1 = number of cells at end of time period.
	Many microorganisms are haploid, so mutations can be expressed immediately.		Where microorganisms divide by binary fission, a single cell will give rise to over 10^6 cells in 20 generations, and to over 10^9 cells in 30 generations. Some mutant white colonies of *Serratia marcescens* will develop if cells which are streaked onto a solid medium are exposed to sublethal doses of ultraviolet light. The nonmutant colonies are red.
	Genes are transferred between microorganisms.	Gene transfers occurring in nature are, in part, responsible for the dramatic increase in antibiotic-resistant organisms that can be found today. Organisms resistant to several antibiotics can even transfer this multiple resistance to other organisms. Transfers occur within the same species and, in some instances, between species (Microbial Genetics, Topic 5.0).	Gene transfers can be studied in the laboratory (Microbial Genetics, Subtopics 5.2–5.5).

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	A wide variety of mutants can be isolated.	Mutant strains of microorganisms are developed and used in industry. Mutants with enzymatic blocks often accumulate large quantities of useful end products. Mutants which are super-producers have been developed to increase the production of antibiotics. Custom-designed organisms are being engineered to perform specific tasks, such as degrading petroleum and synthesizing insulin and interferon.	Pigmentation mutants can readily be observed in colonies on solid media. Phage-resistant mutants develop as colonies on agar plates inoculated with sensitive bacteria and sprayed with phage. Antibiotic-resistant mutants can be isolated on media containing the antibiotic. Nutritionally deficient mutants, called auxotrophs, can be identified by their failure to grow except when a specific growth factor, which they can no longer synthesize, is present in the medium.
	Large populations can be grown in a small volume.		Cell populations of bacteria can exceed 10^9 cells per ml in liquid cultures. Denser populations can be grown on the surface of solid media.
2.0 Genetically Important Macromolecules	The major macromolecules important in the study of genetics are deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein. The genetic information coded in DNA is transcribed into messenger RNA (mRNA), which is then translated into protein.	Exceptions exist, such as in some RNA viruses in which an enzyme, reverse transcriptase, produces a DNA copy of the RNA genome of the virus. **Temin, H.** 1972. RNA-directed DNA synthesis. Sci. Am. **226:**24–33.	
2.1 DNA			
2.11 DNA Structure	The DNA molecule is composed of two complementary chains of nucleotides wound around each other to form a double helix. Each nucleotide consists of a phosphate group, the sugar deoxyribose and one of four bases: adenine (A), guanine (G), thymine (T), and cytosine (C). The sequence of nucleotides on one strand determines the sequence on the second strand. Adenine on one strand pairs by hydrogen bond with thymine; on the other strand guanine pairs with cytosine. The two strands are thus complements of each other.	Watson and Crick, in 1953, deduced the structure of DNA from X-ray crystallographic data. Chargaff's work of the late 1940's and early 1950's showed that the four nucleotides of DNA are not present in equal amounts in DNA and that the amounts vary from one species to another. He also showed that in DNA, the number of moles of adenine equals that of thymine; the number of moles of cytosine equals that of guanine. The base sequence of DNA can be determined. The entire genome of several viruses has been sequenced.	The two strands of a DNA molecule are aligned in an antiparallel manner, i.e., one strand runs in a 3′ to 5′ direction and the other strand runs in a 5′ to 3′ direction. The numbers refer to the carbon atoms in deoxyribose. The base composition of DNA can be determined by its isolation, acid hydrolysis, and then chromatography to separate the bases. Detection and quantitation of these bases yield the concentration of each. The percent guanine plus cytosine (% G+C) content is a convenient description of base composition of DNA.

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	Crick, F. H. C. 1954. The structure of the hereditary material. Sci. Am. **191**:54–61.	**Fiddes, J. C.** 1977. The nucleotide sequence of a viral DNA. Sci. Am. **237**:54–67.	The G+C content of any DNA can be calculated by the following formula: $\% G + C = \frac{[G] + [C]}{[A] + [T] + [C] + [G]} \times 100$ (where each base is measured in moles). The base composition of the DNA determines the temperature at which the two strands separate, this temperature being the melting temperature, or T_m. The higher the % G+C value, the higher the T_m. To determine the buoyant density of a DNA molecule, the CsCl density gradient centrifugation technique is used. The buoyant density of a DNA molecule is a linear function of the ratio of G–C to A–T complementary base pairs.
2.12 DNA Function	DNA has two major functions: to replicate itself and to code for the synthesis of protein. A gene is the sequence of nucleotides which codes for a single protein (or polypeptide). The entire array of genes in an organism comprises its genome.	Khorana and colleagues (1970) succeeded in chemically synthesizing the gene coding for the production of yeast alanine tRNA. **Holley, R. W.** 1966. The nucleotide sequence of a nucleic acid. Sci. Am. **214**:30–39.	Early evidence that DNA is the carrier of genetic information came from several different researchers. The transformation experiments of Griffiths (1928) demonstrated that a transforming principle existed in bacterial cells. Avery, MacLeod and McCarthy (1943–1944), using the same species as Griffiths, demonstrated that DNA was capable of transferring inheritable properties to a cell receiving the DNA. Hershey and Chase (1952) demonstrated that in viral infections of bacteria only DNA, and not protein, entered the bacterial cell to initiate the production of more virus particles. Beadle and Tatum (1941) formulated the "one gene, one enzyme" hypothesis from their studies of the mold *Neurospora*. Their hypothesis states a particular gene is responsible for the production of a particular en-

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			zyme, i.e., each enzyme is coded for by a single gene. **Beadle, G. W.** 1948. The genes of men and molds. Sci. Am. **179**:30–39.
2.13 DNA Replication	The two DNA strands separate at a specific point called the origin. As the strands separate, enzymes catalyze the synthesis of two new strands complementary to each of the original two strands. The two new DNA molecules formed consist of one original DNA strand and its newly synthesized complementary strand. This process of DNA replication is termed "semiconservative."	Replication is a complex process catalyzed by a series of polymerases which require a single-stranded template. The general steps in the process have been identified: DNA unwinds at the origin to form a replication fork and expose single-stranded sections. An enzyme synthesizes a short segment of complementary RNA, about 50 nucleotides long, to part of the exposed single strands of DNA, thus forming DNA-RNA hybrids. Using a specific end (called 3′) of the RNA segment as a primer, another enzyme initiates DNA synthesis in a specific direction along the single-stranded DNA. This process results in a DNA-DNA section attached to the RNA-DNA hybrid section. The primer RNA segment is removed by another enzyme, leaving the single-stranded DNA section. Another enzyme synthesizes DNA complementary to those sections from which the RNA was removed. This produces two DNA-DNA double-stranded sections, each identical to the original DNA section. An enzyme seals any gaps in the DNA strands. The process in steps 1 through 6 is repeated around the circular DNA of bacteria until replication is complete, resulting in two DNA molecules exactly like the original DNA molecule (Fig. 1).	Meselson and Stahl (1958) used *Escherichia coli* DNA and a CsCl density centrifugation technique to demonstrate the semiconservative mode of DNA replication. The *E. coli* cells were grown for 14 generations in ^{15}N medium. ^{15}N is a nonradioactive isotope of nitrogen. If ^{15}N is used to synthesize DNA instead of the usual ^{14}N, the ^{15}N DNA will be denser. By switching the ^{15}N- grown cells to ^{14}N medium for one generation and then isolating the DNA, followed by CsCl density gradient centrifugation, they found the DNA from these cells to be intermediate in density to that of cells grown only in ^{14}N. After a second generation, they found that one-half of the DNA was of intermediate density and one-half of the DNA was the density of that of cell grown only on ^{14}N. These results are consistent with semiconservative replication rather than a model in which the parent DNA is conserved intact, or a model in which the parent DNA is randomly dispersed between the progeny molecules. Cairns (1963) used radioactive thymidine to confirm semiconservative replication in bacterial cells. Photographic emulsions placed over slides spread with *E. coli* cells grown on radioactive thymidine showed blackened grains where radioactive particles

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had exposed the photoemulsion. The replicating DNA was photographed in a configuration that is consistent with semiconservative replication.

Cairns, J. 1966. The bacterial chromosome. Sci. Am. **214**:36–44.

2.2 RNA

2.21 RNA Structure

RNA is a single-stranded chain of nucleotides. The nucleotide subunit of RNA differs from that of DNA in that ribose replaces deoxyribose and uracil replaces thymidine. There are three major types of RNA: mRNA, transfer RNA (tRNA), and ribosomal RNA (rRNA).

mRNA is a single, nonhelical strand. In bacteria, an average mRNA is 900 to 1,500 nucleotides long.

Hurwitz, J., and J. J. Furth. 1962. Messenger RNA. Sci. Am. **206**:41–49.

rRNA constitutes up to 65% of ribosomal mass. The bacterial ribosome has two basic subunits: a 50S unit and a 30S unit. These subunits are composed of rRNA and polypeptides. The intact ribosome has a 70S value. Bacterial ribosomes generally have three different linear, single-stranded molecules of RNA with the following S values: 16S, 5S, 23S. tRNA is of relatively small molecular weight, 23,000 to 28,000, with 75 to 90 nucleotide subunits. The bacterial cells contain about 60 different tRNA species. Eucaryotic cells may have 100 to 200 different tRNA's.

tRNA is folded in a specific manner, resembling a cloverleaf. At one of the "leafs" of the cloverleaf is a region called the anticodon site. This site is three nucleotides long; at this region the anticodon bonds with the codon of the mRNA. At another end of the tRNA molecule is an amino acid bonding site (Microbial Genetics, Subtopic 2.332).

Rich, A., and S. H. Kim. 1978. The three-dimensional structure of transfer RNA. Sci. Am. **238**:52–62.

The three kinds of RNA vary in size. One method of measuring RNA size is by an ultracentrifuge technique developed by Svedberg. The sedimentation-velocity method yields a sedimentation coefficient value for the material being centrifuged at high speeds in a solvent. The sedimentation coefficient is called a Svedberg unit and is denoted by the letter S.

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			Eucaryotic ribosomes of the cytoplasm have four different RNA molecules: 5S, 28S, 7S, 18S. Ribosomes in the mitochondria of eucaryotic cells resemble those of bacteria in S values. Ribosomes from the cytoplasm of eucaryotic cells are larger than those of bacteria. The two subunits are 60S and 40S, and the intact ribosome is 73 to 80S.	
2.22	RNA Function	The three types of RNA have specific functions in protein synthesis. mRNA is translated into protein; it is a transitory molecule coding for the synthesis of one specific protein. tRNA molecules carry amino acids to the ribosome. rRNA interacts with specific proteins to form a structure called the ribosome. The ribosome functions as the site of protein synthesis (Microbial Genetics, Subtopic 2.33).	mRNA molecules of eucaryotic cells have a rather long half-life when compared with those of procaryotic mRNA molecules which have half-lives of a few minutes.	Nirenberg and Matthei (1960) developed a cell-free system for protein synthesis. Included in the test system were bacterial ribosomes, amino acids, ATP, RNA, enzymes and cofactors (Microbial Genetics, Subtopic 2.33).
2.23	RNA Synthesis	RNA molecules are synthesized enzymatically, with one strand of DNA as the template (transcription). Specific genes code for each kind of mRNA, tRNA, and rRNA. mRNA synthesis represents the first step in protein synthesis (Microbial Genetics, Subtopic 2.33).	Hybridization techniques have been used to isolate specific genes. In fact, this technique conveniently selects a gene on the DNA for isolation and study.	Under appropriate laboratory conditions, RNA molecules can form double-stranded structures (hybrids with the DNA sequences from which they were transcribed). The hybridization process involves base pairing between the RNA nucleotides and the DNA nucleotides, adenine on the DNA strand pairing with uracil on the RNA strand.
			Certain antibiotics inhibit RNA synthesis. Actinomycin D binds to DNA so RNA polymerase does not complete synthesis of RNA. Rifamycins inhibit bacterial RNA polymerase activity. **Sobell, H. M.** 1974. How actinomycin binds to DNA. Sci. Am. **231:**82–91.	
2.3	Protein			
2.31	Protein Function	A protein or polypeptide is a linear chain of amino acids joined together by peptide bonds. Proteins have unique, three-dimensional structures determined by their amino acid sequences.	The primary structure of a protein molecule is the sequence of amino acids. **Kendrew, J. C.** 1961. The three-dimensional structure of a protein. Sci. Am. **205:** 96–110.	Amino acid sequences of proteins can be determined in laboratories equipped with sequenators and amino acid analyzers. The three-dimensional structure results from intramolecular bonding, both covalent and

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
			noncovalent. In addition, in some proteins two or more polypeptide chains are associated to form a functional protein.
2.32 Protein Function	Proteins function as enzymes and as structural elements. All the properties of a cell are determined by its proteins.		
2.33 Protein Synthesis	Genetic information flows from the nucleotide sequence of the DNA to the nucleotide sequence of mRNA to the primary structure of the protein. The first part of the process is transcription; the second part is translation (Fig. 2).	The two processes are separated by a nuclear membrane in eucaryotes. Transcription is a nuclear process; translation occurs on the ribosome.	Electron micrographs of procaryotes have shown the two processes of transcription and translation of the same gene occur almost simultaneously. Nirenberg and Matthaei (1961) developed a cell-free system to synthesize polypeptides. This system included ribosomes, amino acids, and a synthetic RNA polymer of known composition used as mRNA. A synthetic RNA with only uracil as the base coded for a polypeptide of only phenylalanine.
2.331 Transcription	The mRNA molecule is synthesized complementary to a gene on one strand of DNA. Transcription begins and ends at specific sites on the DNA. A sequence of three bases of the mRNA is called a codon and specifies one amino acid.	DNA is transcribed by RNA polymerase enzyme which has several subunits. One of the subunits, the sigma factor, is responsible for recognizing the specific DNA site where transcription is initiated. For some genes, this site is on one strand of DNA, and for other genes, this site is on the other strand. In eucaryotes, there are several RNA polymerases which transcribe different kinds of RNA. The complete genetic code can be found in most biochemistry texts.	
2.332 Translation	The mRNA binds to the ribosome, where it is decoded into a sequence of amino acids. Each tRNA molecule functions as an adaptor by binding to a specific amino acid at one end and recognizing and binding to a specific codon in the mRNA on the ribosome. As the mRNA moves along the ribosome successive codons are translated and amino acids polymerize in the proper sequence (Fig. 3).	Translation of mRNA involves four major steps. (i) Activation of tRNA. Each amino acid has a specific enzyme, aminoacyl-tRNA synthetase, which covalently links a specific amino acid to its tRNA. The amino acid-tRNA combination is termed aminoacyl-tRNA. (ii) Initiation of translation. A complex is formed among the first aminoacyl tRNA, the mRNA and the ribosome.	

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TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		(iii) Polymerization of amino acids. A second tRNA binds to the codon adjacent to the initiating tRNA. As a peptide bond forms enzymatically between the two adjacent amino acids, the initiating tRNA discharges its amino acid. Additional tRNA molecules bind in sequence to their appropriate codons and pick up the growing peptide chain in turn. (iv) Termination. The protein chain is completed when the ribosome reaches a codon which codes for no amino acid (a nonsense codon). The activation of tRNA and peptide chain elongation requires energy. **Crick, F. H. C.** 1962. The genetic code. Sci. Am. **207:**66–74. **Nirenberg, M. W.** 1963. The genetic code II. Sci. Am. **208:**80–94. **Crick, F. H. C.** 1966. The genetic code III. Sci. Am. **215:**55–62.	
3.0 Organization of Genetic Material 3.1 Procaryotic Organization			
3.11 Chromosome	The procaryotic chromosome generally exists as a closed circular DNA molecule. The chromosome is not surrounded by a nuclear membrane. No structural proteins have been identified.	A typical bacterial chromosome (*Escherichia coli*) has a molecular weight of $\sim 3 \times 10^9$ and contains 4.5×10^6 nucleotide pairs.	The nuclear region can be seen within the cell, both by staining and by phase microscopy.
		It exists as a very long, looped (supercoiled) molecule. The % G+C of the DNA differs between species. It may vary from ~25 to 70% G+C. The sequence of bases in the DNA may be used as a measure of the relationship of different species. The more identical the sequence, the closer the relationship. DNA replication and cell division may proceed at different rates in bacteria. The average number of chromosomes, therefore,	The circular chromosome may be visualized by electron microscopy. The chromosome may be isolated attached to the bacterial membrane by detergent lysis of the cell, followed by centrifugation.

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
		varies with the growth rate of the cell. The faster the cell grows, the greater the number.	
3.12 Plasmid	A plasmid is an extrachromosomal, closed circular DNA molecule. It may be 0.1 to 10% the size of the cell's genome. Plasmids are not essential for cell growth, but provide the cell with properties that may be useful under certain conditions.	The molecular weight of plasmids varies from $\sim 10^6$ to $>10^8$.	Plasmid DNA may be isolated intact by equilibrium density centrifugation in a salt-dye solution. The closed circular plasmid DNA separates from the linear fragments of the chromosomal DNA.
		Multiple copies of a plasmid may be present in a cell.	Plasmids may be compared in a number of ways. Their molecular weight may be determined or their sequence homology may be compared. They may be cleaved into fragments by enzymes (restriction enzymes) which cut at specific sites. Each plasmid yields a distinct and reproducible fragment pattern.
		Plasmid replication may be under its own control or that of the chromosome. Plasmids may contain genes that control their replication and transfer to other cells. They may also contain structural genes for a variety of proteins. Plasmids may be grouped into classes depending on the gene products for which they code: drug resistance bacteriocin, toxin, degradation of hydrocarbons, and proteins concerned with gene transfer. **Clowes, R. C.** 1973. The molecule of infectious drug resistance. Sci. Am. **228:**18–27. Some plasmids may exist either independently in the cytoplasm or integrated into the bacterial chromosome.	
3.13 Transposon	A transposon is a genetic element (DNA) which cannot exist autonomously but may insert itself at numerous sites either into the chromosome or into plasmids in the cell. It may move (jump) from one DNA molecule to another within the cell.	Transposons carrying a variety of genes have been characterized. These include drug resistance (ampicillin, streptomycin, and kanamycin), toxin synthesis, and β-galactosidase synthesis. **Cohen, N., and J. A. Shapiro.** 1980. Transposable genetic elements Sci. Am. **242:**40–50.	
3.2 Eucaryotic Organization			

TOPICS AND SUBTOPICS		A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
3.21	Nuclear DNA	The majority of genetic material (DNA) of the eucaryote is located in the nucleus. It is found as multiple structures (chromosomes), each containing a large DNA molecule and bound structural and regulatory proteins.	Eucaryotes contain several basic proteins (histones) bound to their DNA. There are five classes of histones which are very closely related in all higher organisms. These help determine the chromosome structure. Acidic proteins bound to the DNA are involved in the regulation of gene expression. The nucleus may contain one (haploid) or two (diploid) copies of each chromosome. Eucaryotic microorganisms contain about 10 times more DNA per haploid genome than procaryotic microorganisms.	The chromosome number can be determined in some cases by staining chromosomes and counting and can be estimated by linkage analysis. The size of the haploid genome can be determined by measurement of the rate at which single strands of DNA reassociate. The kinetics of reassociation can also demonstrate the presence of multiple copies of some sequences in the genome.
3.22	Eucaryotic Organelles	Both mitochondria and chloroplasts contain small DNA molecules similar to those of procaryotes.	DNA of eucaryotic organelles ranges in molecular weight from ~2.5×10^6 to 10^8. Organelle DNA contains genes for rRNA, ribosomal protein, and some, but not all, tRNA's. It also contains genes for some enzymes such as cytochrome oxidase in mitochondria and ribulose diphosphate carboxylase in chloroplasts. The circular organization of the DNA, the lack of bound protein, and the presence of these genes support the theory that eucaryotic organelles evolved from procaryotic organisms. **Goodenough, U. W., and R. P. Levine.** 1970. The genetic activity of mitochondria and chloroplasts. Sci. Am. **223**:22–29.	
3.3	Viral Chromosomes	The genetic information of viruses may be single- or double-stranded DNA, or single- or double stranded RNA, depending on the viral species. No structural proteins are associated with the nucleic acid. The nucleic acid may be either a linear or circular molecule, depending on the virus. **Sinsheimer, R. L.** 1962. Single-stranded DNA. Sci. Am. **207**:109–116.	Some viral DNAs contain modified base residues, such as glucosylated cytosine found in bacteriophage T. Viruses contain many fewer genes than bacteria; their total number ranges from 3 to 250.	
4.0	**Mutation**	A mutation is an inheritable change in the	Mutation events in a population can be	

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	nucleotide sequence of DNA (or RNA, if that is the genetic material).	distinguished from reversible phenotypic changes resulting from environmental changes. Only a few cells in the population in the former instance and all cells in the latter instance show the new phenotype.	
4.1 Types of Mutations	A mutation may change any characteristic of a cell or virus. Mutations are detected by their altered phenotypes resulting from changes in an enzyme or other protein in the mutant cell.	Examples of types of mutations include structural—loss or gain of ability to produce a capsule or flagella; change in colonial morphology; drug or virus resistance—growth in the presence of a drug or virus which kills or inhibits the growth of the parent; loss of catabolic enzymes; nutritional—loss of ability to synthesize a biosynthetic enzyme which produces a requirement for a particular nutrient or growth factor not required by the parent cell (parent is the prototroph and the nutritional mutant derived from it is an auxotroph); and conditional lethal—defect in enzymes required for macromolecular synthesis (essential end product cannot be supplied as in nutritional mutants, and the mutant will grow only if certain environmental conditions are present). These mutants are often temperature sensitive, unable to grow at the higher temperatures at which the parent cells can grow.	Some mutants may be detected by direct observation, as, for example, a change in pigmentation or a change in growth pattern on a differential medium. The replica-plating technique developed by the Lederbergs is particularly useful for detecting and isolating mutants which require a specific growth factor. A velveteen pad is used as an inoculating device to transfer colonies in their exact position from a plate containing an enriched medium to a variety of media lacking particular growth factors. Mutants are identified by their failure to grow on media lacking a particular growth factor. An enrichment technique may be used as a first step in the isolation procedure. The cell population is incubated under conditions in which the parent cell type grows and is thereby killed or eliminated. Under the same conditions, the mutant cell neither grows nor is killed. The remaining cells are then transferred to media in which the mutant can grow. Examples of this technique include the penicillin selection method for bacteria and filtration techniques for mold. Mutations may or may not cause an observable change in phenotype, depending on the extent or site of change. Since more than one codon may represent a particular amino acid, it is possible to have a change in a single base of the DNA molecule resulting in an alternate codon for the same amino acid. It is also possible that slight changes in amino acid sequence in enzymes may have little or no effect on enzyme activity and may go undetected.

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
4.11 Reversion of Mutations	Mutants may revert to their original phenotype.	Restoration of the original phenotype may occur in the following ways: true reversion—the altered nucleotide sequence is restored to the original, or suppression—a mutation occurs elsewhere in the genome that restores the original phenotype. This may be a mutation in the same gene (intragenic) or in another gene (extragenic). A suppressor mutation usually refers to extragenic suppression.	The Ames test is based on the reversion of mutants (Microbial Genetics, Subtopics 4.4).
4.2 Mechanisms of Mutation	The nucleotide sequence of DNA may be changed by substitution of one nucleotide for another or by addition or deletion of one or more nucleotides.	Mutational events involving a change in a single nucleotide or nucleotide pair are called point mutations. Figure 4 illustrates the effect of adding a nucleotide pair, resulting in a type of mutation referred to as a frameshift mutation. Large deletions are not revertible; small deletions may be revertible by suppression.	
4.3 Rates of Mutation	The frequency with which mutations occur at specific sites on the DNA molecule differs. Mutation rate expresses the probability that a cell will undergo mutation at a particular gene. An average frequency of spontaneous mutation at any given gene is 10^{-6}, or one change in a million.	A "spontaneous mutation" arises with no intervention on the part of the investigator. The frequency range for spontaneous mutations is 10^{-4} to 10^{-10}. The probability of two mutational events in one cell is the product of the probability of the two separate mutations. Because of mutations, not all cells arising from a single cell are genetically identical.	
4.4 Mutagenic Agents	Chemical and physical agents which increase the rate of mutation are called mutagenic agents.	Mutagenic agents are used experimentally to increase the frequency of mutation; there is a dose-response effect with any mutagenic agent. No mutagenic agents are known which cause mutations only in particular genes.	The mutagenic activity of ultraviolet (UV) light can be demonstrated by irradiating a bacterial culture (*Serratia marcescens*) and then comparing the frequency of mutations in the irradiated cultures and unirradiated cultures with regard to pigmentation and colony morphology. The Ames test, a screening test for potential carcinogens, is based on the fact that most known carcinogens are mutagenic agents. Since mutagenic agents increase the rate of mutation, they also increase reversion rates. In the Ames test, histidine-requiring *Salmonella* mutants are used; the

Parent Cell—Nonmutant

DNA segment	T C A C G A A T A T G A C T A	
mRNA segment	A G U G C U U A U A C U G A U	(transcription)
AA sequence	ser ala tyr thr asp	(translation)

Mutant Cell—Insertion of Base Pair

DNA segment	T C A C T G A A T A T G A C T A	
mRNA segment	A G U G A C U U A U A C U G A	(transcription)
AA sequence	ser asp leu tyr "Nonsense" chain termination	(translation)

FIG. 4. *Frame shift mutation.*

Categories of mutagenic agents include the following. (i) DNA base analogs substitute for normal bases and result in an increased incidence of incorporation of "wrong" bases. Examples are 5-bromouracil (BU) and 2-aminopurine (AP). (ii) Certain chemicals react with DNA bases in such a way as to change their chemical structure, resulting in a change in hydrogen bonding properties and consequent changes in base pairing, addition or deletion of bases. Examples include nitrous acid, hydroxylamine, monofunctional alkylating agents, such as ethyl methane sulfonate (EMS), bifunctional alkylating agents, nitrogen mustards, mitomycin, and nitrosoguanidine. This last group causes cross-linking between the two DNA strands, prevents unwinding, and may result in deletion of a segment of DNA.

Acridines insert between bases in DNA and cause the addition or deletion of a single base, producing frame-shift mutations.

UV radiation causes formation of covalent bonds between two adjacent thymines on the same strand of DNA (thymine dimer), producing changes in base pairing. In some instances, the actual expression of mutations may depend on the excision and faulty repair of the damaged segment.

Deering, R. A. 1962. Ultraviolet radiation and nucleic acid. Sci. Am. **207**:135–144.

Ionizing radiation (X rays and gamma rays) produces multiple effects on the DNA molecule. Transposons insert into genes, thereby inactivating them (Microbial Genetics, Subtopic 3.13).

rate of reversion to prototrophy is measured in the presence and absence of the chemical being tested. If the chemical is mutagenic, the reversion rate increases.

Devoret, R. 1979. Bacterial tests for potential carcinogens. Sci. Am. **241**:40–49.

4.5 Selection of Mutants

Changes in the environment may give a mutant an advantage so that it will grow

An example of natural selection is the increased incidence of penicillin-resistant

Antibiotic-containing media can be used to select antibiotic-resistant mutants in a

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	faster than the parent and replace it in the population. The change in environment does not cause or induce the mutation but selects for preexisting mutants.	*Staphylococcus* strains after the initiation of penicillin therapy in the 1940s. This mutation was not induced by penicillin, but penicillin acted as a selective agent for preexisting mutants. A population tends to remain genetically stable in the absence of an environmental change selective for mutants; each mutant is in equilibrium at a frequency in the total population proportional to its mutation rate. Since no environment is completely nonselective, cultures maintained over long periods in a laboratory tend to change. Bacteria adapt more readily to new environments than eucaryotic cells because their haploid nature makes possible the immediate expression of mutations, their growth rate is faster, and the large number of cells in a population increases the probability of a wide variety of mutants.	population. The replica-plating technique can be used to demonstrate that the mutants were preexistent and not induced by the antibiotic.
4.6 DNA Repair	Microorganisms have enzymatic mechanisms to repair certain types of DNA. Some mechanisms require visible light.	Photo-repair or light repair mechanisms require visible light. This mechanism repairs UV-induced damage and breaks the covalent bonds joining thymine dimers; it requires a specific enzyme system. Dark repair mechanisms do not require visible light. One type involves specific cellular enzymes which excise the damaged portion of a single DNA strand and other enzymes which then synthesize a segment of DNA complementary to the normal DNA strand. Rare errors may be made in this repair process.	To demonstrate repair mechanisms which require light, UV-irradiated cells may be exposed to light and the frequency of mutations in cells exposed to light may be compared with that of UV-irradiated cells kept in the dark.
5.0 Recombination	Recombination is the joining together of DNA molecules from two parents, which results in traits in the offspring not found together in either of the parents. The process requires the DNA molecules to be homologous to each other.	The frequency of recombination between two genes is proportional to the distance between them. The location of a large number of genes has been mapped on chromosomes of viruses and bacterial and eucaryotic organisms. In *E. coli*, over 600 genes have been mapped. Mapping studies of several viral and bac-	

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			terial chromosomes have shown that many are circular.	
5.1	Viral Genetics	Viruses can undergo recombination.		Infection of the same cell with two viruses, each having a different mutation, results in the release of some virus particles having properties of both infecting particles.
5.11	Virus Replication Lytic	Replication of bacterial, animal, and plant viruses is similar. The nucleic acid of the virus separates from the protein coat and serves as the template for the synthesis of viral components. Host cell enzymes function to supply energy and the building blocks of the virus particles. Multiple viral particles are synthesized and released from the cell. The replication of virulent viruses can be illustrated by the replication of the T-even bacterial virus (bacteriophage or phage) in *E. coli* (Fig. 5). **Stent, G. S.** 1953. The multiplication of bacterial viruses. Sci. Am. 188:36–39.	Animal and bacterial viruses invade cells after binding to specific receptor sites on the walls of the host. This leads to specificity of the virus and the cell it infects. Plant viruses do not attach to specific receptors on the cell walls of the plant and, therefore, have a wide host range. Some viruses are released from the cells they infect by lysing the cells. Other virus particles are extruded from cells without lysing the cell.	Cell lysis can be observed as plaques, or zones of clearing.
5.12	Virus Replication Temperate	Some bacterial viruses have the ability to multiply either as a lytic phage or as a part of the bacterial DNA following the integration of viral DNA into the DNA of the cells they invade. Viruses that have the ability to replicate in this fashion are termed "temperate." The viral nucleic acid (prophage) replicates whenever the bacterial RNA replicates. Bacteria carrying a prophage are termed "lysogenic." The protein of the virus is not synthesized as long as the viral DNA remains integrated in the host DNA. Under certain conditions, the viral DNA becomes excised from the bacterial DNA (induction) and virus particles are formed by the same sequence of reactions involved in lytic replication. (Fig. 6). **Campbell, A.** 1976. How viruses insert their DNA into the DNA of the host cell. Sci. Am. **235:**102–113.	The integrated viral DNA may alter the properties of the host cell. For example, *Corynebacterium diphtheriae* produces a toxin only when lysogenized by a specific phage. Scarlet fever is caused by a lysogenic strain of *Streptococcus pyogenes.* Animal tumor viruses are also capable of integrating their DNA into the host cell DNA. The host cells are transformed by the integrated DNA into cells having many properties that are different from those of the uninfected cells. RNA tumor viruses contain an enzyme that copies the RNA into DNA, which is then integrated into the DNA of the host cell. Herpesvirus DNA may become integrated into the DNA of the host cell after herpes infection. Some temperate phages exist as plasmids as part of their life cycle.	

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5.2	Procaryotic DNA Transfer	A part of the bacterial chromosome may be transferred from donor to recipient cells by three different mechanisms. The three mechanisms are: DNA-mediated transformation, conjugation, and transduction. Once inside the recipient cell, the donor DNA may recombine with the recipient chromosome (Microbial Genetics, Subtopics 5.21–5.23).	Transfer has been demonstrated in only a small minority of bacterial species. The lack of transfer may merely reflect not having the conditions necessary to elicit gene transfer. Procaryotic DNA transfer results in the recombinant remaining haploid, since integration usually occurs quickly after DNA transfer. Occasionally, partial diploids may result if the entering DNA is not integrated.	Transfer can be demonstrated if the donor DNA differs from the recipient DNA and the mixture of the cells is plated on a medium on which only recombinants will be able to form colonies.
5.21	DNA-Mediated Transformation	Short segments of DNA are released from donor cells when they lyse. The DNA can often be taken up and expressed by recipient cells. **Hotchkiss, R. D., and E. Weiss.** 1956. Transformed bacteria. Sci. Am. **195:**48–53.	To take up DNA, the recipient cells must be grown in a certain way that varies from species to species. Such recipient cells are termed competent.	Addition of deoxyribonuclease to the lysed donor cells prevents transfer by degrading the donor DNA. This experiment demonstrates that the donor material is naked (unprotected) DNA.
5.22	Conjugation	DNA is transferred while cells are in physical contact with one another. **Wollmann, E. L., and F. Jacob.** 1956. Sexuality in bacteria. Sci. Am. **195:**109–118.	Cells commonly attach to one another by means of pili. DNA is transferred as a single-stranded molecule only as long as cells remain attached. Transfer of the entire chromosome in *E. coli* requires 90 min, and this occurs very rarely. Conjugation has been observed primarily in gram-negative organisms, although it does occur in the streptococci.	DNA transfer occurs when donor and recipient cells are mixed together and the culture is not disturbed. If the culture is shaken, the cells separate from one another and transfer stops immediately.
5.221	Conjugation F Transfer	Many strains of *E. coli* contain plasmids which transfer from donor to recipient cells. The most thoroughly studied representative plasmid is the F plasmid, which codes for the synthesis of the pili. The chromosome is not transferred when the F plasmid is transferred.	The donor cell is commonly termed the male, or F^+ cell; the recipient is the female, or F^- cell. Transfer of the entire particle occurs within minutes after mixing F^+ and F^- cells. F plasmids can be transferred to species closely related to *E. coli*.	If F^+ and F^- cells are mixed together, the F^- cells are converted to F^+ cells, which can then serve as donors of F plasmids.
5.222	Conjugation Chromosome Transfer	The F particle can exist as an independent, self-replicating entity or it can integrate into the chromosome. When it integrates, the chromosome is capable of being transferred if the donor cell (termed "Hfr") contacts an F^- cell. A genetic element that can	The chromosome is transferred as a linear molecule of single-stranded DNA. Different F particles integrate at different sites, and this determines the order in which the genes are transferred. The integrated F particle is always transferred last. Only a small fraction	[illegible] a population of Hfr cells is mixed with [illegible]s, the ordered transfer of the donor [illegible]osome can be shown by agitating the cells at various times and plating them out on appropriate media for demonstrating transfer of specific genes. Some do-

TOPICS AND SUBTOPICS	A. ESSENTIAL INFORMATION	B. ENRICHMENT INFORMATION	C. PRACTICAL ACTIVITIES
	replicate independently in the cytoplasm or as part of the DNA of the bacterial chromosome is termed an "episome." The process of integration and excision is reversible.	of the recipient cells undergoes recombination with chromosomal DNA.	nor genes will be transferred early, others later.
5.223 Conjugation F' Transfer	When the F particle is excised from the chromosome, a fragment of the chromosome may remain attached to the F particle. This particle with its attached chromosomal DNA is termed an F' particle. It is transferred to F^- cells very rapidly and at a high frequency.	Since only chromosomal DNA adjacent to the site of integration of the F particle can become a part of the F' particle, different F' particles carry different chromosomal genes.	Cells carrying the F' particles can be detected by mixing them with F^- cells. The F' particle is rapidly transferred throughout the F population. This can be determined by plating on a medium which will detect the transfer of the chromosomal genes in the F' particle.
5.23 Transduction	The donor DNA is transferred from donor to recipient cells within the coat of a bacteriophage. Once inside the recipient cell, the bacterial cell, the bacterial genes carried inside the phage coat undergo recombination with the recipient chromosomes. There are two types of transduction, specialized and generalized, depending on the bacterial genes that are transferred. **Zinder, N. D.** 1958. "Transduction" in bacteria. Sci. Am. **195:**109–118.	The bacterial DNA takes the place of some or all phage DNA inside the protein coat of the phage. Thus, the phage is "defective" since it does not have all of the genes required for its own replication.	Recipient cells are transduced when a lysate from a phage-infected culture of donor cells is added to recipient cells. The recipient cells are grown under conditions which minimize lysis and are then plated on a medium which will detect the transfer of genes from donor to recipient cells.
5.231 Specialized Transduction	Specialized transduction results from the transfer of a certain group of genes located next to the site at which temperate phage DNA has become integrated into the donor chromosome. Upon induction, a piece of the donor DNA remains attached to the phage DNA (Fig. 7).	The same phage may transduce different bacterial genes because the phage DNA may become integrated at different sites. There are preferred and less-preferred sites for integration. The bacteriophage lambda and P22 are specialized transducing phages in *E. coli.*	
5.232 Generalized Transduction	In generalized transduction, any segment of the bacterial chromosome can be transferred. The usual case involves a temperate phage which packages a piece of donor DNA inside its protein coat as it goes through its virulent life cycle. This DNA is transferred to a recipient cell when the phage infects (Fig. 8).	In many cases, the coat of the phage is filled completely with bacterial DNA of the donor cell.	
5.24 Plasmid Transfer	An entire plasmid, or parts of it, may be transferred by the same three mechanisms	Plasmids may be transferred between widely different genera. These include *Esch-*	

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	that operate for the transfer of chromosomal genes. The plasmid DNA, however, does not have to be homologous to the recipient DNA.	*erichia*, *Shigella*, *Salmonella*, *Proteus*, *Pseudomonas*, and *Vibrio*. The increase in the incidence of antibiotic-resistant organisms in the hospital environment is due largely to the transfer of plasmids that code for antibiotic resistance into previously sensitive cells. A single plasmid may carry the genes coding for resistance to seven different antibiotics.	
5.3 Recombinant DNA	Recombinant DNA currently refers to two different DNA molecules joined together in vitro. Commonly, a plasmid which can replicate in bacteria is one component of the molecule. The second component is DNA from any source. The hybrid plasmid is then introduced by DNA-mediated transformation into *E. coli*, where it replicates. Thus, the foreign DNA introduced into the plasmid becomes cloned, resulting in the production of many identical copies (Fig. 9).	Restriction enzymes, which recognize specific nucleotide sequences in DNA, are often used to cleave the DNA molecules which are to be joined together. It has been possible to clone eucaryotic genes in *E. coli* and to produce cells of *E. coli* which synthesize insulin and growth hormone. **Cohen, S. N.** 1975. The manipulation of genes. Sci. Am. **233**:24–33. Foreign DNA can also be cloned in lambda phage.	
5.4 Eucaryotic Recombination	There are two means of achieving genetic recombination: two haploid gametes, usually from different parents, fuse to produce a new individual with a combination of traits from each parent; or in meiosis (reduction division), a diploid cell divides so that each nucleus receives only one member of each chromosome pair. During this process, when the chromosome pair aligns, they may exchange pieces. In this way, genes are arranged in new combination or recombine during meiosis.	Eucaryotes exhibit several mating patterns: they may or may not produce specialized mating structures; and in some, but not all cases, they exhibit mating types. A and α in yeast is one of the best known. Special features: fungi can fuse so that nuclei with different genetic complements can exist in the same cytoplasm (heterocaryons). In this case, they may exhibit recombination and segregation during mitotic division by a process called the parasexual cycle. In some protozoans, differentiated nuclei exist; one type is concerned with expression and cell function and the other is involved with sexual reproduction.	Fungi are the most widely used system for genetic analysis of eucaryotes. In the *Ascomycetes*, sexual union occurs within a sac. Fusion of nuclei is followed by meiosis and the products are called ascospores.
		Some eucaryotic microorganisms have both haploid and diploid phases in their life cycles. In the haploid phase, the nuclei contain one copy of each chromosome; diploid nuclei contain two copies of each chromosome and, therefore, of each gene. An orga-	Because of the way in which the chromosomes segregate during nuclear division, chromosome mechanics have been studied by analysis of the ordered ascospores in *Neurospora*.

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		nism can maintain either phase by means of mitotic division; each nucleus, whether haploid or diploid, reproduces exact copies of itself. Mitotic division occurs with no concomitant recombination of genetic material (Fig. 10).	
			By crossing two strains of *Sordaria* which produce different-colored ascospores, the arrangement of spores in each ascus provides immediate linear evidence of the chromosomal events which occurred during meiosis.
6.0 Regulatory Mechanisms	Independent mechanisms exist for regulating gene expression and the activity of preexisting enzymes. Alteration of the environment may reversibly alter phenotype without altering genotype.	Jacob and Monod developed the operon theory which provides an explanation for regulation of gene activity (repression and induction). They also developed the model by which preexisting enzymes in a metabolic pathway are rendered inactive in the presence of the end product of the pathway (feedback inhibition).	An example of an environmental influence is the production of pigment in *Serratia marcescens*. Pigment production occurs only when cultures are incubated at temperatures below 37°C.
6.1 Regulation of Gene Activity	Repression and induction regulate the initiation of gene transcription.	Regulation of gene activity prevents synthesis of needless enzymes. Energy and materials are conserved. The functional state of the repressor is determined by the presence or absence of the specific metabolite. An operon is a cluster of genes which is transcribed as a unit. The operon contains structural genes which code for the production of several enzymes in a metabolic pathway, and an operator region. A regulator gene, often located at a distance from the operon, produces a repressor protein which, when activated, can bind to the operator and block transcription of the structural genes by preventing the binding of RNA polymerase to DNA. The initiation of transcription of all the genes in the operon is thus prevented. Repressor proteins are allosteric; one site attaches to the operator and the other attaches to a specific metabolite. Operons have been found in many bacteria but apparently are rare in eucaryotes.	

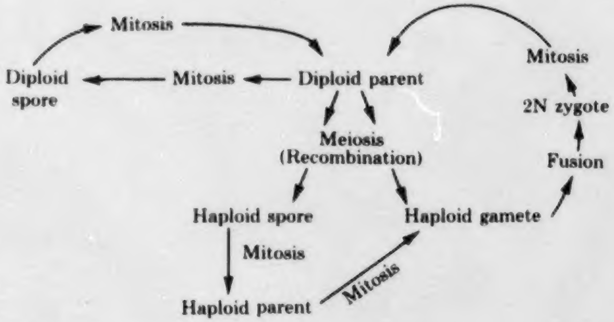
Mitosis
Diploid spore
Mitosis
Diploid parent
Mitosis
2N zygote
Meiosis (Recombination)
Fusion
Haploid spore
Haploid gamete
Mitosis
Haploid parent
Mitosis

FIG. 10. *Generalized eucaryotic life cycle.*

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		Ptashne, M., and W. Gilbert. 1970. Genetic repressors. Sci. Am. **222:**36–44.	
6.11 Repression	In end-product repression, the end product of a biosynthetic pathway blocks the transcription of genes coding for the entire group of enzymes involved in that pathway.	In the absence of the end product, the repressor protein is inactive and cannot attach to the operator, and the operon is turned on. When the end product of the pathway is present, it binds to the repressor protein, changing its conformation, thereby activating it, so that it can bind to the operator and "turn off" transcription. The functional state of the repressor is determined by the presence or absence of the specific metabolite (Fig. 11).	When *E. coli* is grown in a medium containing tryptophan, the organism does not make the enzymes for the biosynthesis of tryptophan. If tryptophan is not present in the medium, the enzymes for tryptophan synthesis are produced until the concentration of tryptophan synthesized by the cell shuts down further enzyme synthesis.
6.12 Induction	In enzyme induction, the substrate in a degradative pathway triggers the transcription of genes coding for enzymes in that pathway.	In inducible systems, the operon is normally turned off because repressor protein is bound to the operator. When the substrate of an inducible pathway is present, it binds to the repressor protein, changing its conformation, thereby inactivating it so that it can no longer bind to the operator. Transcription can then occur. In addition to substrate, cyclic adenosine monophosphate (AMP) and a protein (CAP) are required for transcription (Fig. 12).	*E. coli* produces the enzyme β-galactosidase only when lactose is present in the medium.
6.2 Modification of Enzyme Activity	In feedback inhibition, the end product of a biosynthetic pathway inhibits the activity of the first enyzme in that pathway. Since no product of the first reaction is formed subsequent enzymes in that pathway will have no substrate and the entire pathway will be shut down.	Feedback inhibition conserves energy and materials. Feedback inhibition operates when an enzyme is an allosteric protein. When the end product combines with the allosteric site on that enzyme, its conformation is altered, thus altering its activity (Fig. 13).	Repressible systems, such as the synthesis of tryptophan in *E. coli*, also show feedback inhibition. In the presence of tryptophan, the first enzyme in the biosynthetic pathway for tryptophan synthesis is inhibited by tryptophan.

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